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BIOASSAY OF 5-CHLORO-o-TOLUIDINE FOR POSSIBLE CARCINOGENICITY

CAS No. 95-79-4

NCI-CG-TR-187

U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
Public Health Service
National Institutes of Health

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REPORT ON BIOASSAY OF 5-CHLORO-O-TOLUIDINE FOR POSSIBLE CARCINOGENICITY

Availability

5-Chloro-o-toluidine (CAS 95-79-4) has been tested for cancer-causing activity with rats and mice in the Carcinogenesis Testing Program, Division of Cancer Cause and Prevention, National Cancer Institute. A report is available to the public.

Summary: A bioassay for the possible carcinogenicity of 5-chloro-o-toluidine was conducted using Fischer 344 rats and B6C3F1 mice. Applications of the chemical include use as an intermediate in the manufacture of dyes. 5-Chloro-o-toluidine was administered in the feed, at either of two concentrations, to groups of 50 male and 50 female animals of each species.

Under the conditions of this bioassay, 5-chloro-o-toluidine was carcinogenic to B6C3F1 mice, inducing hemangiosarcomas and hepatocellular carcinomas in both males and females. There was no conclusive evidence of the carcinogenicity of the compound in Fischer 344 rats.

Single copies of the report, Bioassay of 5-Chloro-o-toluidine for Possible Carcinogenicity (T.R. 187), are available from the Office of Cancer Communications, National Cancer Institute, Building 31, Room 10A21, National Institutes of Health, Bethesda, Maryland 20014.

Dated: January 26, 1979

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Director
National Institutes of Health

(Catalogue of Federal Domestic Assistance Program Number 13.393, Cancer Cause and Prevention Research)

BIOASSAY OF

5-CHLORO-o-TOLUIDINE

FOR POSSIBLE CARCINOGENICITY

Carcinogenesis Testing Program
Division of Cancer Cause and Prevention
National Cancer Institute
National Institutes of Health
Bethesda, Maryland 20014

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REPORT ON THE BIOASSAY OF 5-CHLORO-o-TOLUIDINE
FOR POSSIBLE CARCINOGENICITY

CARCINOGENESIS TESTING PROGRAM
DIVISION OF CANCER CAUSE AND PREVENTION
NATIONAL CANCER INSTITUTE, NATIONAL INSTITUTES OF HEALTH

FOREWORD: This report presents the results of the bioassay of 5-chloro-o-toluidine conducted for the Carcinogenesis Testing Program, Division of Cancer Cause and Prevention, National Cancer Institute (NCI), National Institutes of Health, Bethesda, Maryland. This is one of a series of experiments designed to determine whether selected chemicals have the capacity to produce cancer in animals. Negative results, in which the test animals do not have a significantly greater incidence of cancer than control animals, do not necessarily mean the test chemical is not a carcinogen because the experiments are conducted under a limited set of circumstances. Positive results demonstrate that the test chemical is carcinogenic for animals under the conditions of the test and indicate a potential risk to man. The actual determination of the risk to man from animal carcinogens requires a wider analysis.

CONTRIBUTORS: This bioassay of 5-chloro-o-toluidine was conducted by Litton Bionetics, Inc., Kensington, Maryland, initially under direct contract to the NCI and currently under a subcontract to Tracor Jitco, Inc., prime contractor for the NCI Carcinogenesis Testing Program.

The experimental design was determined by the NCI Project Officers, Dr. N. P. Page (1,2), Dr. E. K. Weisburger (1) and Dr. J. H. Weisburger (1,3). The principal investigators for the contract were Dr. F. M. Garner (4) and Dr. B. M. Ulland (4,5). Mr. S. Johnson (4) was the coprincipal investigator for the contract. Animal treatment and observation were supervised by Mr. R. Cypher (4), Mr. D. S. Howard (4) and Mr. H. D. Thornett (4); Mr. H. Paulin (4) analyzed dosed feed mixtures. Ms. J. Blalock (4) was responsible for data collection and assembly.

Histopathologic examinations were performed at Litton Bionetics, Inc. (4), and the slides for mice were reviewed by Dr. A. DePaoli (4). The slides for rats were reviewed at Experimental Pathology Laboratories, Inc. (6). The rat pathology narrative was written by Dr. J. F. Hardisty (6), the mouse pathology narrative was written by Dr. A. DePaoli (4), and the diagnoses included in this report represent the interpretation of these pathologists. Histopathology findings and reports were reviewed by Dr. R. L. Schueler (7).

Compilation of individual animal survival, pathology and summary tables was performed by EG&G Mason Research Institute (8); the statistical analysis was performed by Mr. R. M. Helfand (9) and Dr. J. P. Dirkse, III (10), using methods selected for the Carcinogenesis Testing Program by Dr. J. J. Gart (11).

This report was prepared at METREK, a Division of The MITRE Corporation (9) under the direction of the NCI. Those responsible for this report at METREK are the project coordinator, Dr. L. W. Thomas (9), task leader Ms. P. Walker (9), senior biologist Mr. M. Morse (9), biochemist Mr. S. C. Drill (9), chemist Dr. N. Zimmerman (9), and technical editor Ms. P. A. Miller (9). The final report was reviewed by members of the participating organizations.

The following other scientists at the National Cancer Institute were responsible for evaluating the bioassay experiment, interpreting the results, and reporting the findings: Dr. K. C. Chu (1), Dr. C. Cueto, Jr. (1), Dr. J. F. Douglas (1), Dr. R. A. Griesemer (1), Dr. T. E. Hamm (1), Dr. W. V. Hartwell (1), Dr. M. H. Levitt (1), Dr. H. A. Milman (1), Dr. T. W. Orme (1), Dr. A. R. Patel (1), Dr. S. F. Stinson (1), Dr. J. M. Ward (1), and Dr. C. E. Whitmire (1).

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SUMMARY

A bioassay for the possible carcinogenicity of 5-chloro-o-toluidine was conducted using Fischer 344 rats and B6C3F1 mice. 5-Chloro-o-toluidine was administered in the feed, at either of two concentrations, to groups of 50 male and 50 female animals of each species. Twenty animals of each sex and species were placed on test as controls. The high and low dietary concentrations of 5-chloro-o-toluidine were 5000 and 2500 ppm for rats and 4000 and 2000 ppm for mice. The compound was administered for 78 weeks to both rats and mice, followed by an observation period of up to 26 weeks for rats and 13 weeks for mice.

There were significant positive associations between the concentrations of 5-chloro-o-toluidine administered and mortality among male and female mice, but not among rats of either sex. Adequate numbers of animals in all groups survived sufficiently long to be at risk from late-developing tumors. Distinct mean body weight depression was apparent when dosed female rats and dosed mice of both sexes were compared to their controls, indicating that the concentrations administered to these animals in this bioassay may have approximated the maximum tolerated concentrations. Since no mean body weight depression, relative to controls, no significantly accelerated mortality, and no signs of toxicity other than fatty metamorphosis of the liver were associated with administration of 5-chloro-o-toluidine to male rats, it is possible that these animals may have been able to tolerate a higher dietary concentration.

There was a significant positive association between the concentration of 5-chloro-o-toluidine administered to male rats and the incidence of adrenal pheochromocytomas in these animals; however, neither of the Fisher exact comparisons was significant. None of the other statistical tests for tumors at any site in male or female rats indicated a significant positive association between dosage and incidence.

In mice of both sexes there were significant positive associations between concentration administered and the incidence of hemangiosarcomas. In addition, the high dose to control Fisher exact comparisons for both sexes were significant. The Cochran-Armitage tests were also significant and positive for the incidences of hepatocellular carcinomas in both sexes of mice. For males and females, the high dose to control Fisher exact comparisons were significant, and for females the low dose to control comparison was also significant.

Under the conditions of this bioassay, 5-chloro-o-toluidine was carcinogenic to B6C3F1 mice, inducing hemangiosarcomas and hepatocellular carcinomas in both males and females. There was no conclusive evidence of the carcinogenicity of the compound in Fischer 344 rats.

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I. INTRODUCTION

5-Chloro-o-toluidine (Figure 1) (NCI No. C02051), an aromatic amine and dye intermediate, was selected for bioassay by the National Cancer Institute because of the high incidence of bladder cancer observed among workers in the dye manufacturing industry (Anthony and Thomas, 1970; Wynder et al., 1963). Aromatic amines are one of several classes of compounds thought to be responsible for this increased cancer risk (Clayson and Garner, 1976).

The Chemical Abstracts Service (CAS) Ninth Collective Index (1977) name for this compound is 5-chloro-2-methylbenzenamine.* It is also called p-chloro-o-aminotoluene; 4-chloro-2-aminotoluene; 2-amino-4-chlorotoluene; o-amino-p-chlorotoluene; 5-chloro-2-methylaniline; 2-methyl-5-chloroaniline; 1-amino-2-methyl-5-chlorobenzene; 1-amino-3-chloro-6-methylbenzene; Fast Red KB Base; Azogene Fast Red KB; Brentamine Fast Red KB Base; Naphthosol Fast Red KB Base; and Naphthanil Red KB Base.

Both 5-chloro-o-toluidine and its hydrochloride salt are used as C.I. (Colour Index) Azoic Diazo Component 32, a component of azoic dyes (Society of Dyers and Colourists, 1956b). Azoic dyes are formed on textile fibers, especially cellulose, by the reaction of selected diazo and coupling components. C.I. Azoic Diazo Component 32 is also recommended for dyeing silk and nylon (Society of Dyers and Colourists, 1956a).

*The CAS registry number is 95-79-4.

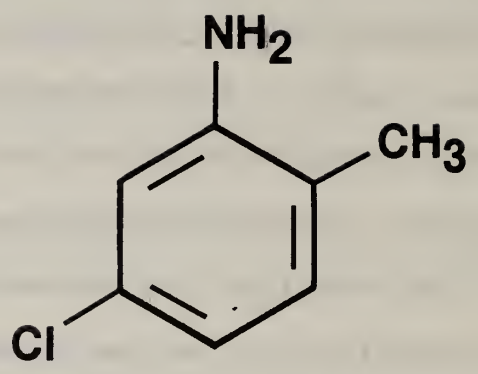


FIGURE 1
CHEMICAL STRUCTURE OF 5-CHLORO-o-TOLUIDINE

Specific production data for 5-chloro-o-toluidine are not available; however, this compound and/or its hydrochloride salt are currently produced or sold in commercial quantities (in excess of 1000 pounds or $1000 in value annually) as C.I. Azoic Diazo Component 32 by one U.S. company (U.S. International Trade Commission, 1977). U.S. imports of 5-chloro-o-toluidine amounted to 42,163 pounds in 1974 (U.S. International Trade Commission, 1976).

The potential for exposure to 5-chloro-o-toluidine is greatest for workers in the chemical and dye manufacturing and textile industries.

II. MATERIALS AND METHODS

A. Chemicals

Technical-grade 5-chloro-o-toluidine was purchased from E.I. duPont de Nemours & Company, Wilmington, Delaware. Chemical analysis was performed by Litton Bionetics, Inc., Kensington, Maryland. The experimentally determined refractive index was $n_D^{20} = 1.5831$. Thin-layer chromatography was performed utilizing two solvent systems (i.e., benzene:methanol and diethyl ether:ethyl acetate:acetic acid). Each plate, visualized with ultraviolet and visible light, iodine vapor, and ferric chloride-potassium ferricyanide spray, revealed one single spot. Only one homogeneous peak was observed using gas chromatography. The results of infrared and nuclear magnetic resonance analyses were consistent with those expected based on the structure of the compound.

Throughout this report, the term 5-chloro-o-toluidine is used to represent this technical-grade material.

B. Dietary Preparation

The basal laboratory diet for both dosed and control animals consisted of Wayne Lab-Blox® meal (Allied Mills, Inc., Chicago, Illinois). 5-Chloro-o-toluidine was administered to the dosed animals as a component of the diet.

The chemical was removed from its container and a proper amount was blended with an aliquot of the feed using a mortar and pestle. Once visual homogeneity was attained, the mixture was placed in a 6

kg capacity Patterson-Kelley standard model twin-shell stainless steel V-blender along with the remainder of the feed to be prepared. After 20 minutes of blending, the mixtures were placed in double plastic bags and stored in the dark at 4°C. The mixture was prepared once weekly.

C. Animals

The two animal species, Fischer 344 rats and B6C3F1 mice, used in the carcinogenicity bioassay were obtained through contracts of the Division of Cancer Treatment, National Cancer Institute. Rats were supplied by A. R. Schmidt, Madison, Wisconsin; Laboratory Supply Company, Inc., Indianapolis, Indiana; and Charles River Breeding Laboratories, Inc., Wilmington, Massachusetts. Mice were supplied by A. R. Schmidt and Charles River Breeding Laboratories, Inc.

Rats and mice, approximately 4 weeks old when received, were examined and any obviously ill or runted animals were killed. The remaining animals were quarantined for 2 weeks prior to initiation of test. Animals which did not manifest clinical signs of disease were assigned to groups and distributed among cages so that the average body weight per cage was approximately equal for a given species and sex.

D. Animal Maintenance

Animals were housed by species in rooms maintained at 22° to 26°C and 45 to 55 percent relative humidity. Incoming air was filtered through HEPA filters (Flanders Filters, McLean, Virginia)

at a rate of 12 to 15 complete changes of room air per hour. Fluorescent lighting was provided 8 hours per day (9:00 a.m. to 5:00 p.m.).

Animals were housed by species in separate rooms. Rats were housed four per cage by sex and mice five per cage by sex. Polycarbonate cages (Lab Products, Inc., Garfield, New Jersey) suspended from aluminum racks, were used for dosed and control animals. Racks were fitted with a continuous piece of stainless steel mesh over which a sheet of filter paper was firmly secured. Filter paper was changed at 2-week intervals, when the racks were sanitized. Clean cages and hardwood chip bedding (Ab-sorb-dri®, Wilner Wood Products Company, Norway, Maine) were provided twice weekly.

Acidulated water (pH 2.5) was supplied ad libitum to animals in water bottles which were changed and washed twice weekly. Sipper tubes were washed at weekly intervals. During the period of chemical administration, dosed and control animals received ad libitum treated or untreated Wayne Lab-Blox® meal as appropriate. The feed was supplied in hanging stainless steel hoppers which were refilled three times per week and sanitized weekly.

Dosed and control rats were housed in a room with other rats receiving diets containing* 3-chloro-p-toluidine (95-74-9); 2-nitro-p-phenylenediamine (5307-14-2); and nitrofen (1836-75-5).

*CAS registry numbers are given in parentheses.

Dosed and control mice were housed in a room with mice receiving diets containing Michler's ketone (90-94-8); 4,4'-methylenebis (N,N-dimethyl)benzenamine (101-61-1); p-chloroaniline (106-47-8); N-phenyl-p-phenylenediamine hydrochloride (2198-59-6); 1-phenyl-2-thiourea (103-85-5); trimethylthiourea (2489-77-2); dibutyltin diacetate (1067-33-0); and 2-nitro-p-phenylenediamine (5307-14-2).

E. Selection of Initial Concentrations

To establish the concentrations of 5-chloro-o-toluidine for administration to dosed animals in the chronic studies, subchronic toxicity tests were conducted with both rats and mice. Animals of each species were distributed among six groups, each consisting of five males and five females. 5-Chloro-o-toluidine was incorporated into the basal laboratory diet and supplied ad libitum to five of the six rat groups in concentrations of 315, 680, 1465, 3155 and 6800 ppm and to five of the six mouse groups in concentrations of 255, 550, 1180, 2550 and 5500 ppm. The remaining group of each species served as a control group, receiving only the basal laboratory diet.

The dosed dietary preparations were administered for a period of 4 weeks, followed by a 2-week observation period during which all animals were fed the basal laboratory diet. Individual body weights were recorded twice weekly throughout the study. Upon termination of the study all survivors were euthanized and necropsied.

The following table indicates the mean body weight gain, relative to controls, survival and incidence of abnormal signs observed in each of the rat groups at the end of the subchronic test.

RAT SUBCHRONIC STUDY RESULTS

ppm	Mean Body Weight Gain (%)[a]		Survival[b]		Observation of Abnormal Signs[b]	
	Males	Females	Males	Females	Males	Females
5800	- 35	- 21	5/5	5/5	5/5[c,d]	5/5[c]
3155	+ 23	- 3	5/5	5/5	5/5[c,d]	5/5[c]
1465	- 19	+ 3	5/5	5/5	5/5[c,d]	5/5[c]
680	+ 8	+ 5	5/5	5/5	5/5[c,d]	5/5[c]
315	+ 18	+ 4	5/5	5/5	5/5[c,d]	5/5[c]
0	--	--	5/5	5/5	0/5	0/5

The high concentration selected for administration to dosed rats in the chronic bioassay was 5000 ppm.

The following table indicates the mean body weight gain, relative to controls, and survival observed in each of the mouse groups at the end of the subchronic test.

MOUSE SUBCHRONIC STUDY RESULTS

ppm	Mean Body Weight Gain (%)[a]		Survival[b]	
	Males	Females	Males	Females
5500	--	- 5	0/5	5/5
2550	+ 7	- 3	5/5	5/5
1180	+ 5	0	5/5	5/5
550	+ 3	- 6	5/5	5/5
255	+16	- 2	5/5	5/5
0	--	--	5/5	5/5

[a] + is indicative of mean body weight gain greater than that of controls.
- is indicative of mean body weight gain less than that of controls.
[b] Number of animals observed/number of animals originally in group.
[c] These rats had mottled livers.
[d] These rats had spotted kidneys.

No other abnormalities which could be attributed to administration of the compound were observed. The high concentration selected for administration to dosed mice in the chronic bioassay was 4000 ppm.

F. Experimental Design

The experimental design parameters for the chronic study (species, sex, group size, concentrations administered, and duration of treated and untreated observation periods) are summarized in Tables 1 and 2.

Rats were approximately 6 weeks old at the time the test was initiated and all were placed on test on the same day. Dosed rats were supplied with diets containing 5000 and 2500 ppm 5-chloro-o-toluidine for 78 weeks followed by an observation period of up to 26 weeks, when no test chemicals were used. Throughout this report those rats receiving the former concentration are referred to as the high dose groups and those receiving the latter concentration are referred to as the low dose groups.

All mice were approximately 6 weeks old at the time the test was initiated and were placed on test on the same day. Dosed mice were supplied with diets containing 4000 and 2000 ppm 5-chloro-o-toluidine for 78 weeks followed by a 13-week observation period, when no test chemicals were used. Throughout this report those mice receiving the former concentration are referred to as the high dose groups and those receiving the latter concentration are referred to as the low dose groups.

TABLE 1

DESIGN SUMMARY FOR FISCHER 344 RATS
5-CHLORO-o-TOLUIDINE FEEDING EXPERIMENT

	INITIAL GROUP SIZE	5-CHLORO-o-TOLUIDINE CONCENTRATION[a]	OBSERVATION PERIOD TREATED (WEEKS)	OBSERVATION PERIOD UNTREATED (WEEKS)
MALE				
CONTROL	20	0	0	104
LOW DOSE	50	2500	78	
		0		26
HIGH DOSE	50	5000	78	
		0		25
FEMALE				
CONTROL	20	0	0	104
LOW DOSE	50	2500	78	
		0		26
HIGH DOSE	50	5000	78	
		0		25

[a]Concentrations given in parts per million.

TABLE 2

DESIGN SUMMARY FOR B6C3F1 MICE
5-CHLORO-o-TOLUIDINE FEEDING EXPERIMENT

	INITIAL GROUP SIZE	5-CHLORO-o-TOLUIDINE CONCENTRATION[a]	OBSERVATION PERIOD TREATED (WEEKS)	OBSERVATION PERIOD UNTREATED (WEEKS)
MALE				
CONTROL	20	0	0	91
LOW DOSE	50	2000	78	
		0		13
HIGH DOSE	50	4000	78	
		0		13
FEMALE				
CONTROL	20	0	0	91
LOW DOSE	50	2000	78	
		0		13
HIGH DOSE	50	4000	78	
		0		13

[a]Concentrations given in parts per million.

G. Clinical and Histopathologic Examinations

Animals were weighed immediately prior to initiation of the experiment. Body weights of rats were recorded once a week for the first 6 weeks, every 2 weeks for the next 12 weeks, once a month for the next 32 weeks, and at 8-week intervals for the remainder of the bioassay. Body weights of mice were recorded once a week for the first 6 weeks, every 2 weeks for the next 12 weeks, and at monthly intervals thereafter. Animals were inspected twice daily. Food consumption data were collected at monthly intervals from 20 percent of the animals in each group.

All moribund animals, animals that developed large, palpable masses that jeopardized their health, or animals that survived until the end of the bioassay were euthanized using carbon dioxide inhalation. Necropsies were immediately performed on these animals and on all animals found dead during the bioassay. Gross and microscopic examinations were performed on all major tissues, organs, and gross lesions taken from sacrificed animals and, whenever possible, from animals found dead.

Tissues were preserved in a 10 percent neutral buffered formalin solution, embedded in paraffin, sectioned, and stained with hematoxylin and eosin prior to microscopic examination.

Slides were prepared from the following tissues: skin, subcutaneous tissue, lungs and bronchi, trachea, bone marrow, spleen, lymph nodes, thymus, heart, salivary gland, liver, gallbladder (mice),

pancreas, esophagus, stomach, small intestine, large intestine, kidney, urinary bladder, pituitary, adrenal, thyroid, parathyroid, testis, prostate, brain, uterus, mammary gland, and ovary.

A few tissues were not examined for some animals, particularly for those that died early. Also, some animals were missing, cannibalized, or judged to be in such an advanced state of autolysis as to preclude histopathologic interpretation. Thus, the number of animals for which particular organs, tissues, or lesions were examined microscopically varies and does not necessarily represent the number of animals that were recorded in each group at the time that the test was initiated.

H. Data Recording and Statistical Analyses

Pertinent data on this experiment have been recorded in an automatic data processing system, the Carcinogenesis Bioassay Data System (Linhart et al., 1974). The data elements include descriptive information on the chemicals, animals, experimental design, clinical observations, survival, body weight, and individual pathologic results, as recommended by the International Union Against Cancer (Berenblum, 1969). Data tables were generated for verification of data transcription and for statistical review.

These data were analyzed using the statistical techniques described in this section. Those analyses of the experimental results that bear on the possibility of carcinogenicity are discussed in the statistical narrative sections.

Probabilities of survival were estimated by the product-limit procedure of Kaplan and Meier (1958) and are presented in this report in the form of graphs. Animals were statistically censored as of the time that they died of other than natural causes or were found to be missing; animals dying from natural causes were not statistically censored. Statistical analyses for a possible dose-related effect on survival used the method of Cox (1972) when testing two groups for equality and used Tarone's (1975) extensions of Cox's methods when testing a dose-related trend. One-tailed P-values have been reported for all tests except the departure from linearity test, which is only reported when its two-tailed P-value is less than 0.05.

The incidence of neoplastic or nonneoplastic lesions has been given as the ratio of the number of animals bearing such lesions at a specific anatomic site (numerator) to the number of animals in which that site was examined (denominator). In most instances, the denominators included only those animals for which that site was examined histologically. However, when macroscopic examination was required to detect lesions prior to histologic sampling (e.g., skin or mammary tumors), or when lesions could have appeared at multiple sites (e.g., lymphomas), the denominators consist of the numbers of animals necropsied.

The purpose of the statistical analyses of tumor incidence is to determine whether animals receiving the test chemical developed a significantly higher proportion of tumors than did the control

animals. As a part of these analyses, the one-tailed Fisher exact test (Cox, 1970, pp. 48-52) was used to compare the tumor incidence of a control group to that of a group of treated animals at each dose level. When results for a number of treated groups, k, are compared simultaneously with those for a control group, a correction to ensure an overall significance level of 0.05 may be made. The Bonferroni inequality (Miller, 1966, pp. 6-10) requires that the P-value for any comparison be less than or equal to 0.05/k. In cases where this correction was used, it is discussed in the narrative section. It is not, however, presented in the tables, where the Fisher exact P-values are shown.

The Cochran-Armitage test for linear trend in proportions, with continuity correction (Armitage, 1971, pp. 362-365), was also used when appropriate. Under the assumption of a linear trend, this test determined if the slope of the dose-response curve is different from zero at the one-tailed 0.05 level of significance. Unless otherwise noted, the direction of the significant trend was a positive dose relationship. This method also provides a two-tailed test of departure from linear trend.

A time-adjusted analysis was applied when numerous early deaths resulted from causes that were not associated with the formation of tumors. In this analysis, deaths that occurred before the first tumor was observed were excluded by basing the statistical tests on animals that survived at least 52 weeks, unless a tumor was found at the

anatomic site of interest before week 52. When such an early tumor was found, comparisons were based exclusively on animals that survived at least as long as the animal in which the first tumor was found. Once this reduced set of data was obtained, the standard procedures for analyses of the incidence of tumors (Fisher exact tests, Cochran-Armitage tests, etc.) were followed.

When appropriate, life-table methods were used to analyze the incidence of tumors. Curves of the proportions surviving without an observed tumor were computed as in Saffiotti et al. (1972). The week during which animals died naturally or were sacrificed was entered as the time point of tumor observation. Cox's methods of comparing these curves were used for two groups; Tarone's extension to testing for linear trend was used for three groups. The statistical tests for the incidence of tumors which used life-table methods were one-tailed and, unless otherwise noted, in the direction of a positive dose relationship. Significant departures from linearity ($P < 0.05$, two-tailed test) were also noted.

The approximate 95 percent confidence interval for the relative risk of each dosed group compared to its control was calculated from the exact interval on the odds ratio (Gart, 1971). The relative risk is defined as p_t/p_c where p_t is the true binomial probability of the incidence of a specific type of tumor in a treated group of animals and p_c is the true probability of the spontaneous incidence of the same type of tumor in a control group. The hypothesis of equality

between the true proportion of a specific tumor in a treated group and the proportion in a control group corresponds to a relative risk of unity. Values in excess of unity represent the condition of a larger proportion in the treated group than in the control.

The lower and upper limits of the confidence interval of the relative risk have been included in the tables of statistical analyses. The interpretation of the limits is that in approximately 95 percent of a large number of identical experiments, the true ratio of the risk in a treated group of animals to that in a control group would be within the interval calculated from the experiment. When the lower limit of the confidence interval is greater than one, it can be inferred that a statistically significant result (a $P < 0.025$ one-tailed test when the control incidence is not zero, $P < 0.050$ when the control incidence is zero) has occurred. When the lower limit is less than unity but the upper limit is greater than unity, the lower limit indicates the absence of a significant result while the upper limit indicates that there is a theoretical possibility of the induction of tumors by the test chemical which could not be detected under the conditions of this test.

III. CHRONIC TESTING RESULTS: RATS

A. Body Weights and Clinical Observations

The mean body weights of dosed male rats were slightly lower than that of the controls from week 15 until week 80. Distinct mean body weight depression, in comparison with their controls, was observed in dosed female rats throughout the bioassay (Figure 2).

No other clinical signs were recorded.

B. Survival

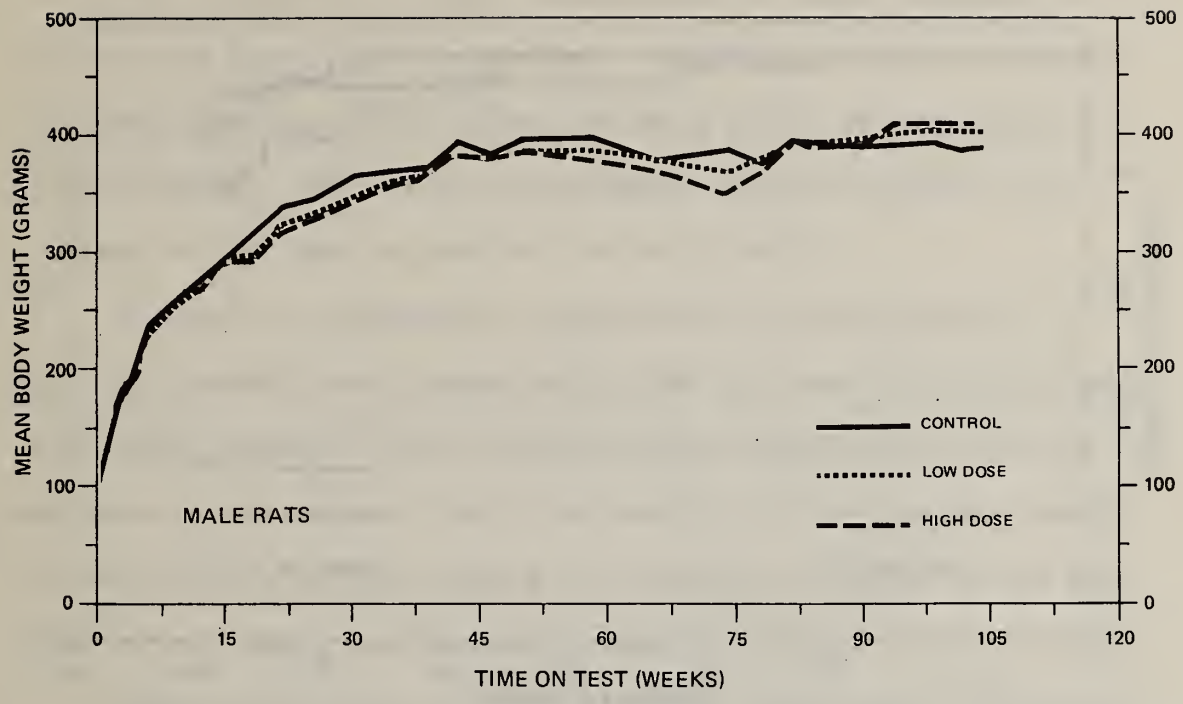
The estimated probabilities of survival for male and female rats in the control and 5-chloro-o-toluidine-dosed groups are shown in Figure 3. The Tarone test for association between dosage and mortality was not significant for either males or females.

There were adequate numbers of male rats at risk from late-developing tumors, as 94 percent (47/50) of the high dose, 78 percent (39/50) of the low dose, and 85 percent (17/20) of the controls survived on test until the termination of the study.

There were adequate numbers of female rats at risk from late-developing tumors, as 92 percent (46/50) of the high dose, 80 percent (40/50) of the low dose, and 75 percent (15/20) of the controls survived on test until the termination of the study.

C. Pathology

Histopathologic findings on neoplasms in rats are summarized in Appendix A (Tables A1 and A2); findings on nonneoplastic lesions are summarized in Appendix C (Tables C1 and C2).



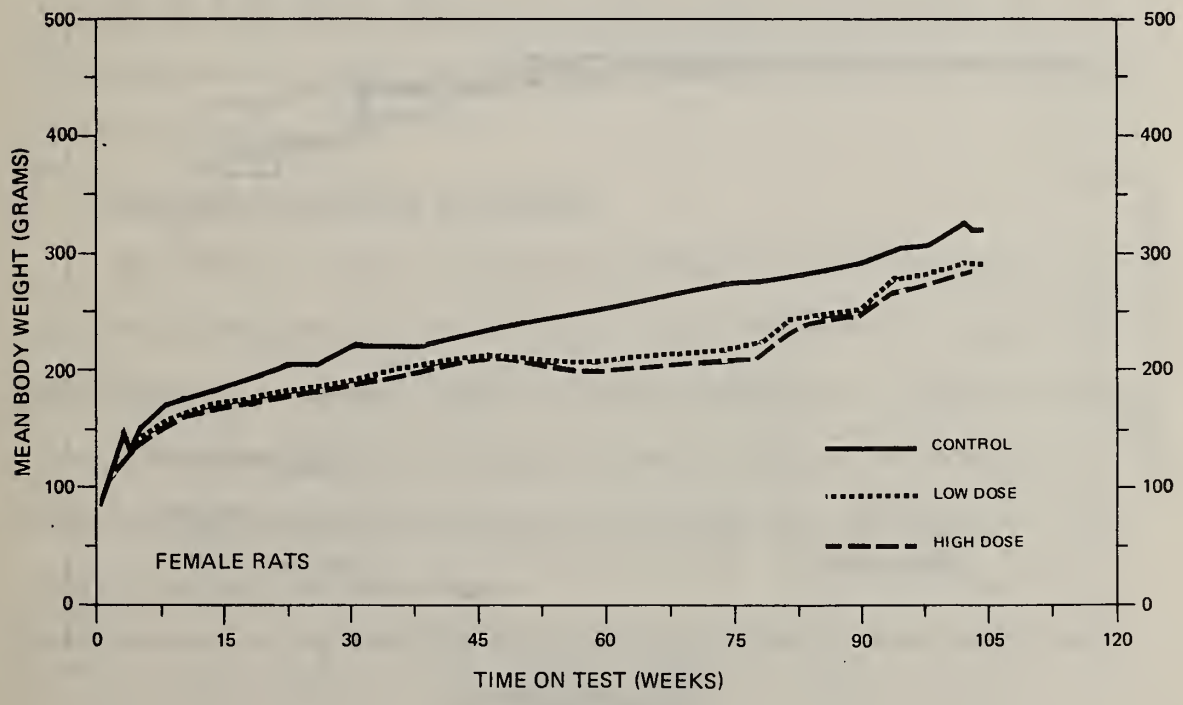
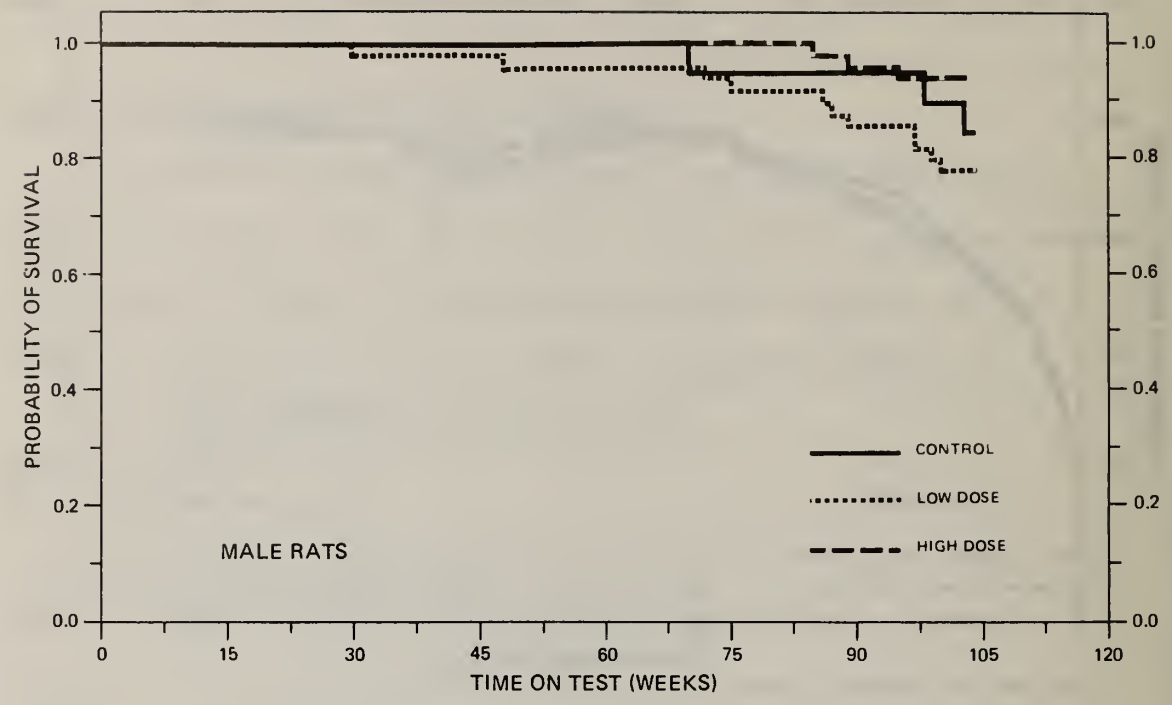


FIGURE 2
GROWTH CURVES FOR 5-CHLORO-o-TOLUIDINE CHRONIC STUDY RATS



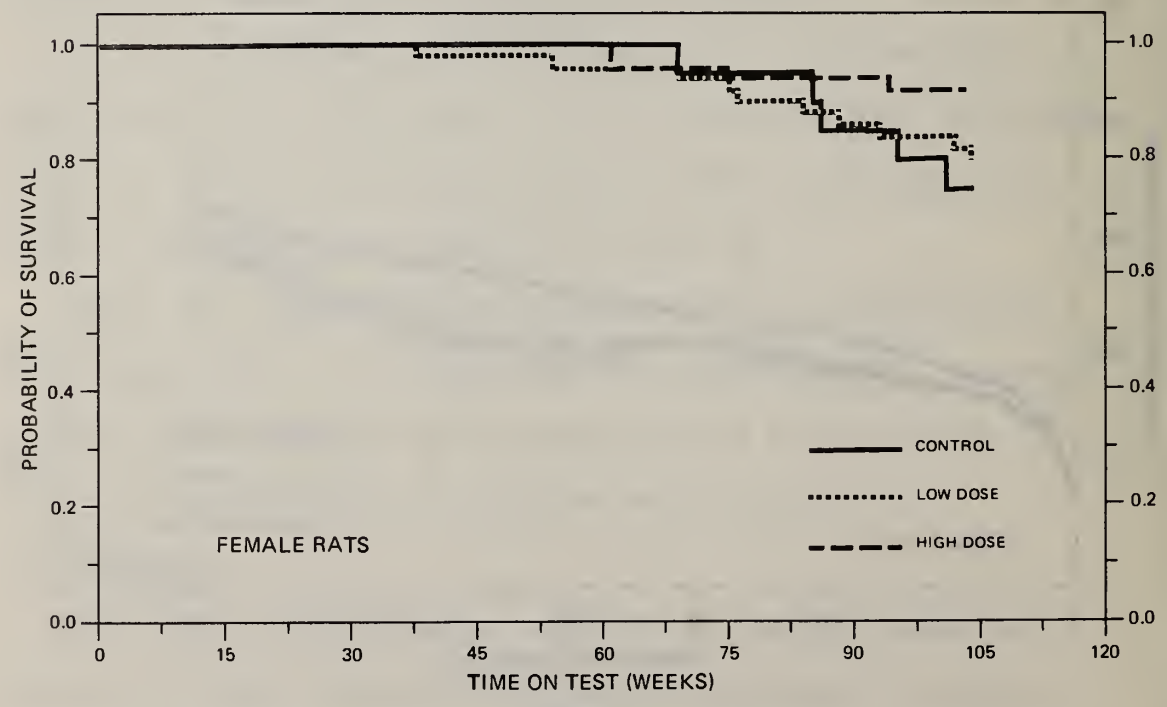


FIGURE 3
SURVIVAL COMPARISONS OF 5-CHLORO-o-TOLUIDINE CHRONIC STUDY RATS

Most neoplasms were seen at approximately equal incidence in the control and dosed rats. A few types of neoplasms occurred only, or with increased incidence, in rats of dosed groups as compared with control groups. The nature and incidence of these neoplasms are similar to that seen in aged rats of this strain.

A variety of inflammatory, degenerative and proliferative lesions commonly seen in aged Fischer 344 rats were observed in dosed and control animals. None of these lesions appeared to be related to exposure to the compound, with the exception of fatty metamorphosis of the liver, which was observed in increased incidences in the dosed rats of both sexes when compared to control groups.

Based on the results of this pathology examination, the administration of 5-chloro-o-toluidine, at the dosage levels used, was not carcinogenic to Fischer 344 male and female rats under the conditions of this bioassay.

D. Statistical Analyses of Results

The results of the statistical analyses of tumor incidence in rats are summarized in Tables 3 and 4. The analysis is included for every type of malignant tumor in either sex where at least two such tumors were observed in at least one of the control or 5-chloro-o-toluidine-dosed groups and where such tumors were observed in at least 5 percent of the group.

TABLE 3

ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS AT
SPECIFIC SITES IN MALE RATS TREATED WITH 5-CHLORO-o-TOLUIDINE[a]

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Skin and Subcutaneous Tissue: Fibroadenoma[b]	1/20(0.05)	2/50(0.04)	6/50(0.12)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	0.800	2.400
Lower Limit	---	0.045	0.325
Upper Limit	---	46.273	108.021
Weeks to First Observed Tumor	103	100	103
Hematopoietic System: Leukemia or Malignant Lymphoma[b]	2/20(0.10)	4/50(0.08)	1/50(0.02)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	0.800	0.200
Lower Limit	---	0.128	0.004
Upper Limit	---	8.436	3.681
Weeks to First Observed Tumor	98	86	103
Adrenal: Pheochromocytoma[b]	0/20(0.00)	2/49(0.04)	7/48(0.15)
P Values[c]	P = 0.019	N.S.	N.S.
Relative Risk (Control)[d]	---	Infinite	Infinite
Lower Limit	---	0.125	0.843
Upper Limit	---	Infinite	Infinite
Weeks to First Observed Tumor	---	97	103

TABLE 3 (CONTINUED)

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Pancreatic Islets: Islet-Cell Adenoma[b]	0/20(0.00)	4/45(0.09)	0/45(0.00)
P Values[c]	N.S.	N.S.	N.S.
Departure from Linear Trend[e]	P = 0.018	---	---
Relative Risk (Control)[d]	---	Infinite	---
Lower Limit	---	0.429	---
Upper Limit	---	Infinite	---
Weeks to First Observed Tumor	---	100	---
Testis: Interstitial-Cell Tumor[b]	18/20(0.90)	47/50(0.94)	47/50(0.94)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	1.044	1.044
Lower Limit	---	0.918	0.918
Upper Limit	---	1.246	1.246
Weeks to First Observed Tumor	103	72	89
Body Cavities: Mesothelioma[b]	1/20(0.05)	0/50(0.00)	3/50(0.06)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	0.000	1.200
Lower Limit	---	0.000	0.106
Upper Limit	---	7.475	61.724
Weeks to First Observed Tumor	104	---	103

TABLE 3 (CONCLUDED)

[a]Treated groups received doses of 2500 or 5000 ppm in feed.

[b]Number of tumor-bearing animals/number of animals examined at site (proportion).

[c]The probability level for the Cochran-Armitage test is given beneath the incidence of tumors in the control group when $P < 0.05$; otherwise, not significant (N.S.) is indicated. The probability level for the Fisher exact test for the comparison of a treated group with the control group is given beneath the incidence of tumors in the treated group when $P < 0.05$; otherwise, not significant (N.S.) is indicated. For both Cochran-Armitage and Fisher exact tests a negative designation (N) indicates a lower incidence in the treated group(s) than in the control group.

[d]The 95% confidence interval on the relative risk of the treated group to the control group.

[e]The probability level of the test for departure from linear trend is given beneath the control group when $P < 0.05$.

TABLE 4

ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS AT
SPECIFIC SITES IN FEMALE RATS TREATED WITH 5-CHLORO-o-TOLUIDINE[a]

TOPOGRAPHY:MORHPOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Pituitary: Chromophobe Carcinoma or Chromophobe Adenoma[b]	5/20(0.25)	6/43(0.14)	7/46(0.15)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	0.558	0.609
Lower Limit	---	0.166	0.196
Upper Limit	---	2.084	2.196
Weeks to First Observed Tumor	85	75	103
Thyroid: C-Cell Carcinoma[b]	1/16(0.06)	0/41(0.00)	5/47(0.11)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	0.000	1.702
Lower Limit	---	0.000	0.217
Upper Limit	---	7.266	78.693
Weeks to First Observed Tumor	104	---	103
Thyroid: C-Cell Carcinoma or C-Cell Adenoma[b]	2/16(0.13)	1/41(0.02)	6/47(0.13)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	0.195	1.021
Lower Limit	---	0.004	0.213
Upper Limit	---	3.555	9.783
Weeks to First Observed Tumor	104	104	103

TABLE 4 (CONTINUED)

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Mammary Gland: Fibroadenoma[b]	1/20(0.05)	10/50(0.20)	5/50(0.10)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	4.000	2.000
Lower Limit	---	0.642	0.249
Upper Limit	---	169.457	92.596
Weeks to First Observed Tumor	104	102	103
Mammary Gland: Fibroadenoma, Adenocarcinoma NOS or Cystadenoma NOS[b]	2/20(0.10)	10/50(0.20)	6/50(0.12)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	2.000	1.200
Lower Limit	---	0.488	0.243
Upper Limit	---	17.808	11.574
Weeks to First Observed Tumor	101	102	103
Uterus: Endometrial Stromal Polyp[b]	0/18(0.00)	4/49(0.08)	1/47(0.02)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	Infinite	Infinite
Lower Limit	---	0.357	0.021
Upper Limit	---	Infinite	Infinite
Weeks to First Observed Tumor	---	104	103

TABLE 4 (CONCLUDED)

[a]Treated groups received doses of 2500 or 5000 ppm in feed.

[b]Number of tumor-bearing animals/number of animals examined at site (proportion).

[c]The probability level for the Cochran-Armitage test is given beneath the incidence of tumors in the control group when $P < 0.05$; otherwise, not significant (N.S.) is indicated. The probability level for the Fisher exact test for the comparison of a treated group with the control group is given beneath the incidence of tumors in the treated group when $P < 0.05$; otherwise, not significant (N.S.) is indicated. For both Cochran-Armitage and Fisher exact tests a negative designation (N) indicates a lower incidence in the treated group(s) than in the control group.

[d]The 95% confidence interval on the relative risk of the treated group to the control group.

The Cochran-Armitage test indicated a significant (P = 0.019) positive association between dose and the incidence of pheochromocytomas of the adrenal in male rats. However, neither of the Fisher exact tests was significant.

None of the other statistical tests for any site indicated a significant association between dose and tumor incidence. Thus, there was not sufficient evidence to indicate the carcinogenicity of 5-chloro-o-toluidine in Fischer 344 rats of either sex.

To provide additional insight into the possible carcinogenicity of this compound, 95 percent confidence intervals on the relative risk have been estimated and entered in the tables based upon the observed tumor incidence rates. In many of the intervals shown in Tables 3 and 4, the value one is included; this indicates the absence of statistically significant results. It should also be noted that many of the confidence intervals have an upper limit greater than one, indicating the theoretical possibility of tumor induction in rats by 5-chloro-o-toluidine that could not be established under the conditions of this test.

IV. CHRONIC TESTING RESULTS: MICE

A. Body Weights and Clinical Observations

Distinct and consistent dose-related mean body weight depression was apparent in male mice. The mean body weights of dosed female mice were consistently lower than that for the controls after week 20; however, no dose-related effect was observed (Figure 4).

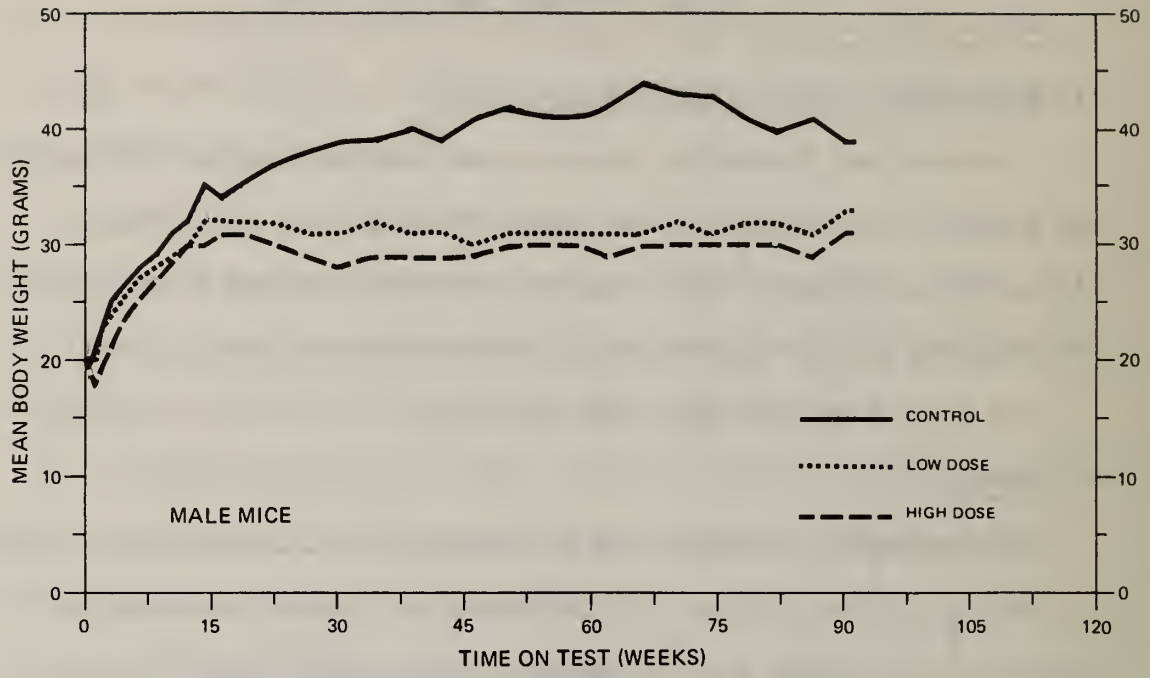
No other clinical signs were recorded.

B. Survival

The estimated probabilities of survival for male and female mice in the control and 5-chloro-o-toluidine-dosed groups are shown in Figure 5. The Tarone test for association between dosage and mortality was significant for both males ($P < 0.001$) and females ($P = 0.039$). In addition, the Cox test was significant when comparing the high dose males with their controls ($P = 0.001$).

There were adequate numbers of male mice at risk from late-developing tumors, as all males survived on test for at least 64 weeks and 46 percent (23/50) of the high dose, 82 percent (41/50) of the low dose and 95 percent (19/20) of the controls survived on test until the termination of the study. Two high dose males were missing in week 34.

There were adequate numbers of female mice at risk from late-developing tumors, as 62 percent (31/50) of the high dose, 86 percent (43/50) of the low dose and 90 percent (18/20) of the controls survived on test until the termination of the study.



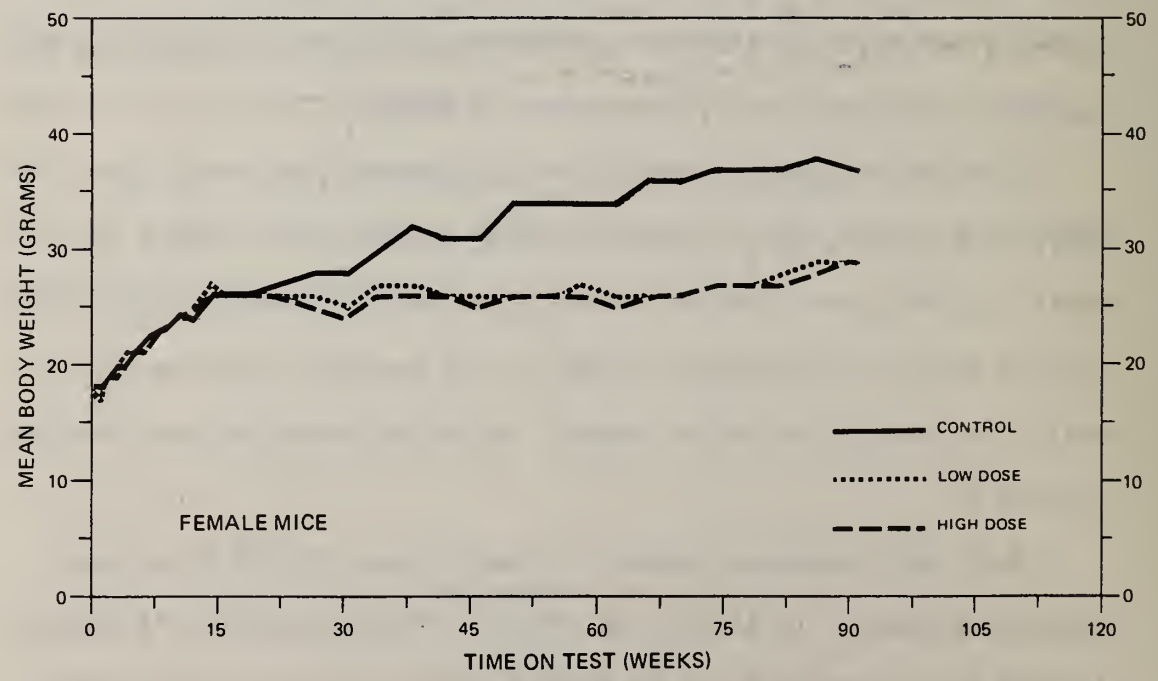
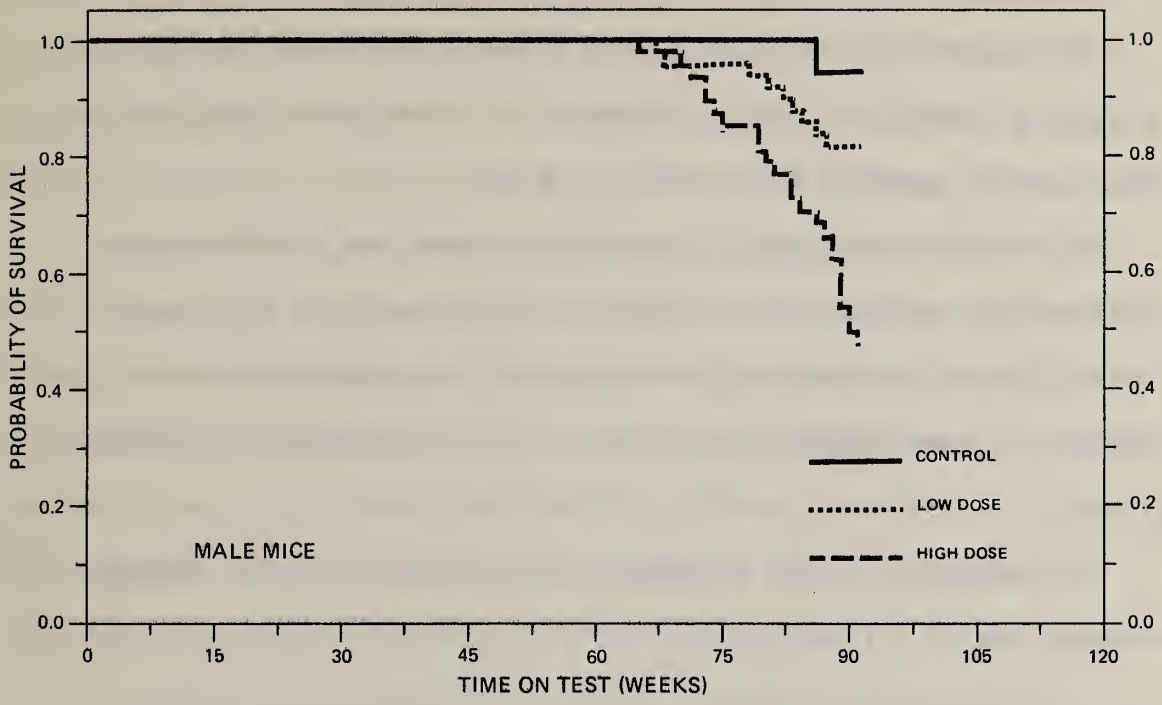


FIGURE 4
GROWTH CURVES FOR 5-CHLORO-o-TOLUIDINE CHRONIC STUDY MICE



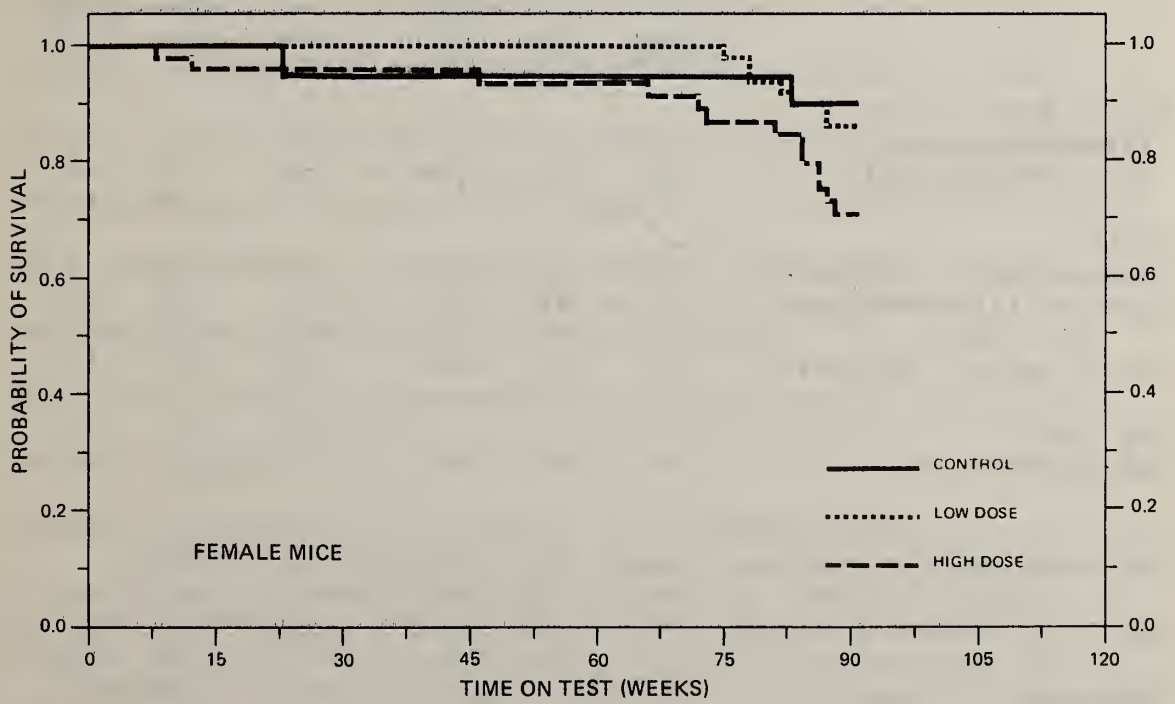


FIGURE 5
SURVIVAL COMPARISONS OF 5-CHLORO-o-TOLUIDINE CHRONIC STUDY MICE

C. Pathology

Histopathologic findings on neoplasms in mice are summarized in Appendix B (Tables B1 and B2); findings on nonneoplastic lesions are summarized in Appendix D (Tables D1 and D2).

An increased incidence of hemangiosarcomas and hepatocellular neoplasms was observed in the dosed groups as compared with the control groups. In addition, a variety of other tumors commonly observed in aged B6C3F1 mice occurred in both the control and dosed groups.

The incidence of the hepatocellular neoplasms and the hemangiosarcomas was as follows:

	MALES			FEMALES		
	CONTROL	LOW DOSE	HIGH DOSE	CONTROL	LOW DOSE	HIGH DOSE
No. of Animals with Tissues Examined Histopathologically	(20)	(50)	(47)	(20)	(50)	(43)
LIVER						
Hepatocellular Adenoma	0	1	2	0	2	5
Hepatocellular Carcinoma	4	19	25	0	19	26
No. of Animals Necropsied	(20)	(50)	(48)	(20)	(50)	(43)
ALL SITES						
Hemangiosarcoma	1	11	37	0	6	22

The hepatocellular neoplasms ranged from grossly evident nodular masses to lesions discernable only on microscopic examination. The neoplasms, irrespective of size, were irregular in shape. Adenomas generally were smaller, occurring within the confines of a liver

lobule. They compressed adjacent liver parenchyma and were made up of a relatively uniform population of hepatocytes which differed from normal hepatocytes by their increased cytoplasmic basophilia, occasional increased size and failure to form uniform hepatic plates.

Hepatocellular carcinomas were usually larger than hepatocellular adenomas and were composed of variably sized cells showing varying degrees of cytomegaly and cellular atypia. These cells were arranged in sheets, with trabecular or pseudoacinar patterns. Tumor necrosis was not uncommon, particularly in larger specimens. Atypical mitoses, local invasion and/or metastases, usually pulmonary, verified the malignant nature of these lesions.

The hemangiosarcomas varied in size from microscopic foci to grossly evident small red nodules. While a few of these lesions seemed to originate in the spleen, perirenal tissue and skeletal muscle, most lesions in male mice developed in the periepidydimal fat or adjacent pelvic tissues and in females in the periuterine pelvic fat. Histologically, the hemangiosarcomas varied considerably but all demonstrated some degree of vascular pattern. The smallest and apparently earliest lesions were characterized by foci of ectatic vascular channels filled with blood, some being partially or completely occluded by fibrin thrombi. Such channels were lined for the most part by normal-appearing endothelium. However, the neoplastic nature of these lesions was indicated by the presence of foci of larger spindle-shaped epithelioid cells lining some of the

channels. Inflammatory cells, primarily neutrophils, were not uncommon in these early lesions. Most lesions, particularly the larger ones, were composed almost entirely of these atypical endothelial cells. In some specimens, these cells became highly pleomorphic, ranging from spindle-shaped to irregularly round to multinucleated giant cells. In these more anaplastic lesions, neoplastic cells occurred in solid sheets, the vascular nature of the lesion being masked. Mitotic figures, even in the highly anaplastic lesions, usually were very few to nonexistent. The highly malignant nature of these lesions was evident in the tendency toward local invasion and metastases most common to the lung, heart and spleen.

A number of degenerative, proliferative, and inflammatory changes were encountered in dosed and control animals, which are commonly observed in aging B6C3F1 mice. A few of these lesions may be related to compound administration. Such lesions and their incidences are as follows:

	MALES			FEMALES		
	CONTROL	LOW DOSE	HIGH DOSE	CONTROL	LOW DOSE	HIGH DOSE
No. of Animals with Tissues Examined Histopathologically	(20)	(50)	(47)	(20)	(50)	(43)
LIVER						
Cytoplasmic Change	0	18	11	0	10	7
Hyperplasia	0	4	7	0	0	0
BONE MARROW	(19)	(47)	(45)	(19)	(48)	(37)
Myelosclerosis	0	0	0	7	42	32
KIDNEY	(20)	(50)	(47)	(19)	(48)	(43)
Glomerulonephritis	0	2	11	0	0	3

The hepatocytic cytoplasmic change and hyperplasia may represent "preneoplastic" compound-induced changes. The marked increase of myelosclerosis in the dosed versus the control females suggested amplification of this process in the dosed groups. The glomerulonephritis was usually mild. The glomerular changes were characterized by hypercellularity and irregular thickening of basement membrane. While a specific diagnosis requires thin sections and/or electron microscopy, the changes observed are consistent with a membranoproliferative lesion.

Based on the results of this pathology examination, 5-chloro-o-toluidine was carcinogenic to B6C3F1 mice, inducing hemangiosarcomas and hepatocellular adenomas and hepatocellular carcinomas under the conditions of this bioassay.

D. Statistical Analyses of Results

The results of the statistical analyses of tumor incidence in mice are summarized in Tables 5 and 6. The analysis is included for every type of malignant tumor in either sex where at least two such tumors were observed in at least one of the control or 5-chloro-o-toluidine-dosed groups and where such tumors were observed in at least 5 percent of the group.

In male mice the Cochran-Armitage test indicated a significant ($P < 0.001$) positive association between dose and the incidence of hemangiosarcomas. This was supported by a significant ($P < 0.001$) positive Fisher exact high dose to control comparison. The test for

TABLE 5

ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS AT
SPECIFIC SITES IN MALE MICE TREATED WITH 5-CHLORO-o-TOLUIDINE[a]

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Lung: Alveolar/Bronchiolar Carcinoma or Alveolar/Bronchiolar Adenoma[b]	2/20(0.10)	2/50(0.04)	2/48(0.04)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	0.400	0.417
Lower Limit	---	0.032	0.033
Upper Limit	---	5.277	5.490
Weeks to First Observed Tumor	86	91	90
All Sites: Hemangiosarcoma[b]	1/20(0.05)	11/50(0.22)	37/48(0.77)
P Values[c]	P < 0.001	N.S.	P < 0.001
Departure from Linear Trend[e]	P = 0.038	---	---
Relative Risk (Control)[d]	---	4.400	15.417
Lower Limit	---	0.722	3.072
Upper Limit	---	184.752	569.778
Weeks to First Observed Tumor	91	80	67
Liver: Hepatocellular Carcinoma[b]	4/20(0.20)	19/50(0.38)	25/47(0.53)
P Values[c]	P = 0.007	N.S.	P = 0.011
Relative Risk (Control)[d]	---	1.900	2.660
Lower Limit	---	0.752	1.106
Upper Limit	---	6.909	9.228
Weeks to First Observed Tumor	86	65	67

TABLE 5 (CONCLUDED)

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Liver: Hepatocellular Carcinoma or Hepatocellular Adenoma[b]	4/20(0.20)	20/50(0.40)	27/47(0.57)
P Values[c]	P = 0.003	N.S.	P = 0.005
Relative Risk (Control)[d]	---	2.000	2.872
Lower Limit	---	0.799	1.210
Upper Limit	---	7.225	9.830
Weeks to First Observed Tumor	86	65	67

[a]Treated groups received doses of 2000 or 4000 ppm in feed.

[b]Number of tumor-bearing animals/number of animals examined at site (proportion).

[c]The probability level for the Cochran-Armitage test is given beneath the incidence of tumors in the control group when $P < 0.05$; otherwise, not significant (N.S.) is indicated. The probability level for the Fisher exact test for the comparison of a treated group with the control group is given beneath the incidence of tumors in the treated group when $P < 0.05$; otherwise, not significant (N.S.) is indicated. For both Cochran-Armitage and Fisher exact tests a negative designation (N) indicates a lower incidence in the treated group(s) than in the control group.

[d]The 95% confidence interval on the relative risk of the treated group to the control group.

[e]The probability level of the test for departure from linear trend is given beneath the control group when $P < 0.05$.

TABLE 6

ANALYSES OF THE INCIDENCE OR PRIMARY TUMORS AT
SPECIFIC SITES IN FEMALE MICE TREATED WITH 5-CHLORO-o-TOLUIDINE[a]

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
All Sites: Hemangiosarcoma[b]	0/20(0.00)	6/50(0.12)	22/43(0.51)
P Values[c]	P < 0.001	N.S.	P < 0.001
Relative Risk (Control)[d]	---	Infinite	Infinite
Lower Limit	---	0.667	3.487
Upper Limit	---	Infinite	Infinite
Weeks to First Observed Tumor	---	91	46
Lung: Alveolar/Bronchiolar Carcinoma or Alveolar/Bronchiolar Adenoma[b]	1/20(0.05)	0/48(0.00)	3/43(0.07)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	0.000	1.395
Lower Limit	---	0.000	0.123
Upper Limit	---	7.780	71.517
Weeks to First Observed Tumor	91	---	86
Hematopoietic System: Leukemia or Malignant Lymphoma[b]	2/20(0.10)	6/50(0.12)	4/43(0.09)
P Values[c]	N.S.	N.S.	N.S.
Relative Risk (Control)[d]	---	1.200	0.930
Lower Limit	---	0.243	0.149
Upper Limit	---	11.574	9.757
Weeks to First Observed Tumor	83	83	88

TABLE 6 (CONCLUDED)

TOPOGRAPHY:MORPHOLOGY	CONTROL	LOW DOSE	HIGH DOSE
Liver: Hepatocellular Carcinoma[b]	0/20(0.00)	19/50(0.38)	26/43(0.60)
P Values[c]	P < 0.001	P < 0.001	P < 0.001
Relative Risk (Control)[d]	---	Infinite	Infinite
Lower Limit	---	2.548	4.183
Upper Limit	---	Infinite	Infinite
Weeks to First Observed Tumor	---	75	66
Liver: Hepatocellular Carcinoma or Hepatocellular Adenoma[b]	0/20(0.00)	21/50(0.42)	31/43(0.72)
P Values[c]	P < 0.001	P < 0.001	P < 0.001
Relative Risk (Control)[d]	---	Infinite	Infinite
Lower Limit	---	2.840	5.076
Upper Limit	---	Infinite	Infinite
Weeks to First Observed Tumor	---	75	66

[a]Treated groups received doses of 2000 or 4000 ppm in feed.

[b]Number of tumor-bearing animals/number of animals examined at site (proportion).

[c]The probability level for the Cochran-Armitage test is given beneath the incidence of tumors in the control group when P < 0.05; otherwise, not significant (N.S.) is indicated. The probability level for the Fisher exact test for the comparison of a treated group with the control group is given beneath the incidence of tumors in the treated group when P < 0.05; otherwise, not significant (N.S.) is indicated. For both Cochran-Armitage and Fisher exact tests a negative designation (N) indicates a lower incidence in the treated group(s) than in the control group.

[d]The 95% confidence interval on the relative risk of the treated group to the control group.

departure from linear trend was also significant. The Cochran-Armitage test also indicated a significant positive association between dose and the incidence of hepatocellular carcinomas ($P = 0.007$) and between dose and the combined incidence of hepatocellular carcinomas or hepatocellular adenomas ($P = 0.003$). These were both supported by significant ($P = 0.011$ and $P = 0.005$, respectively) positive Fisher exact high dose to control comparisons.

In female mice significant results were obtained at the same sites as for males. The Cochran-Armitage test indicated a significant ($P < 0.001$) positive association between dose and the incidence of hemangiosarcomas. A significant ($P < 0.001$) positive Fisher exact high dose to control comparison supported this finding. The Cochran-Armitage test again indicated significant positive associations between dose and the incidence of hepatocellular carcinomas ($P < 0.001$) and between dose and the combined incidence of hepatocellular carcinomas or hepatocellular adenomas ($P < 0.001$). In both cases the Fisher exact high dose to control comparisons (both with $P < 0.001$) as well as the low dose to control comparisons (both with $P < 0.001$) indicated significant positive results.

Based on these statistical results, 5-chloro-o-toluidine was carcinogenic to B6C3F1 mice of both sexes under the conditions of this bioassay.

V. DISCUSSION

There were significant positive associations between the concentrations of 5-chloro-o-toluidine administered and mortality among male and female mice, but not among rats of either sex. Adequate numbers of animals in all groups survived sufficiently long to be at risk from late-developing tumors. Distinct mean body weight depression was apparent when dosed female rats and dosed mice of both sexes were compared to their controls, indicating that the concentrations administered to these animals in this bioassay may have approximated the maximum tolerated concentrations. Since no mean body weight depression, relative to controls, no significantly accelerated mortality, and no signs of toxicity other than fatty metamorphosis of the liver were associated with administration of 5-chloro-o-toluidine to male rats, it is possible that these animals may have been able to tolerate a higher dietary concentration.

There was a significant positive association between the concentration of 5-chloro-o-toluidine administered to male rats and the incidence of adrenal pheochromocytomas in these animals; however, neither of the Fisher exact comparisons was significant. None of the other statistical tests for tumors at any site in male or female rats indicated a significant positive association between dosage and incidence.

In mice of both sexes there were significant positive associations between the concentration administered and the incidence of

hemangiosarcomas (i.e., 1/20, 11/50, and 37/48 in the control, low dose, and high dose males, respectively, and 0/20, 6/50, and 22/43 in the control, low dose, and high dose females). In addition, the high dose to control Fisher exact comparisons for both sexes were significant. The Cochran-Armitage tests were also significant and positive for the incidences of hepatocellular carcinomas in both sexes (i.e., 4/20, 19/50, and 25/47 in the control, low dose, and high dose males, respectively, and 0/20, 19/50, and 26/43 in the control, low dose, and high dose females). For males and females, the high dose to control Fisher exact comparisons were significant, and for females the low dose to control comparison was also significant.

Under the conditions of this bioassay, 5-chloro-o-toluidine was carcinogenic to B6C3F1 mice, inducing hemangiosarcomas and hepatocellular carcinomas in both males and females. There was no conclusive evidence of the carcinogenicity of the compound in Fischer 344 rats.

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APPENDIX A

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN RATS TREATED WITH 5-CHLORO-o-TOLUIDINE

TABLE A1
SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE RATS TREATED WITH
5-CHLORO-o-TOLUIDINE

	CONTROL(UNTR) 11-1135	LOW DOSE 11-1133	HIGH DOSE 11-1131
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS NECROPSIED	20	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY**	20	50	50
INTEGUMENTARY SYSTEM			
*SKIN	(20)	(50)	(50)
SQUAMOUS CELL CARCINOMA			1 (2%)
BASAL-CELL TUMOR			1 (2%)
FIBROMA			1 (2%)
*SUBCUT TISSUE	(20)	(50)	(50)
ADENOCARCINOMA, NOS	1 (5%)		
FIBROMA	1 (5%)	2 (4%)	5 (10%)
FIBROSARCOMA			2 (4%)
RESPIRATORY SYSTEM			
#LUNG	(20)	(49)	(50)
ADENOCARCINOMA, NOS, METASTATIC	1 (5%)		
ALVEOLAR/BRONCHIOLAR ADENOMA			1 (2%)
ALVEOLAR/BRONCHIOLAR CARCINOMA	1 (5%)	1 (2%)	
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(20)	(50)	(50)
MALIGNANT LYMPHOMA, NOS	1 (5%)		1 (2%)
LEUKEMIA,NOS	1 (5%)	2 (4%)	
MONOCYTIC LEUKEMIA		2 (4%)	
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#SALIVARY GLAND	(19)	(45)	(47)
ADENOMA, NOS			1 (2%)

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED
**EXCLUDES PARTIALLY AUTOLYZED ANIMALS

TABLE A1 (CONTINUED)

	CONTROL (UNTR) 11-1135	LOW DOSE 11-1133	HIGH DOSE 11-1131
#LIVER	(20)	(48)	(50)
HEPATOCELLULAR CARCINOMA		1 (2%)	1 (2%)
#STOMACH	(20)	(50)	(50)
ADENOCARCINOMA, NOS		1 (2%)	
URINARY SYSTEM			
NONE			
ENDOCRINE SYSTEM			
#PITUITARY	(18)	(48)	(48)
CHROMOPHOBE ADENOMA	1 (6%)	2 (4%)	1 (2%)
MIXED TUMOR, BENIGN		1 (2%)	
#ADRENAL	(20)	(49)	(48)
PHEOCHROMOCYTOMA		2 (4%)	7 (15%)
#THYROID	(18)	(44)	(24)
C-CELL CARCINOMA		2 (5%)	1 (4%)
#PANCREATIC ISLETS	(20)	(45)	(45)
ISLET-CELL ADENOMA		4 (9%)	
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(20)	(50)	(50)
FIBROADENOMA			1 (2%)
#TESTIS	(20)	(50)	(50)
INTERSTITIAL-CELL TUMOR	18 (90%)	47 (94%)	47 (94%)
NERVOUS SYSTEM			
#BRAIN	(20)	(49)	(49)
EPENDYMOMA	1 (5%)		
SPECIAL SENSE ORGANS			
NONE			

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE A1 (CONTINUED)

	CONTROL (UNTR) 11-1135	LOW DOSE 11-1133	HIGH DOSE 11-1131
MUSCULOSKELETAL SYSTEM			
*SKELETAL MUSCLE	(20)	(50)	(50)
SARCOMA, NOS			1 (2%)
BODY CAVITIES			
*BODY CAVITIES	(20)	(50)	(50)
MESOTHELIOMA, NOS	1 (5%)		2 (4%)
*TUNICA VAGINALIS	(20)	(50)	(50)
MESOTHELIOMA, NOS			1 (2%)
ALL OTHER SYSTEMS			
*MULTIPLE ORGANS	(20)	(50)	(50)
SARCOMA, NOS		1 (2%)	
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	20	50	50
NATURAL DEATH@	2	9	1
MORIBUND SACRIFICE	1	2	2
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	17	39	47
ANIMAL MISSING			

@ INCLUDES AUTOLYZED ANIMALS

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE A1 (CONCLUDED)

	CONTROL (UNTR) 11-1135	LOW DOSE 11-1133	HIGH DOSE 11-1131
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	20	49	47
TOTAL PRIMARY TUMORS	26	68	75
TOTAL ANIMALS WITH BENIGN TUMORS	18	48	47
TOTAL BENIGN TUMORS	2)	58	65
TOTAL ANIMALS WITH MALIGNANT TUMORS	5	10	6
TOTAL MALIGNANT TUMORS	5	10	7
TOTAL ANIMALS WITH SECONDARY TUMORS#	1		
TOTAL SECONDARY TUMORS	1		
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT	1		3
TOTAL UNCERTAIN TUMORS	1		3
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			

* PRIMARY TUMORS: ALL TUMORS EXCEPT SECONDARY TUMORS
\# SECONDARY TUMORS: METASTATIC TUMORS OR TUMORS INVASIVE INTO AN ADJACENT ORGAN

TABLE A2
SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE RATS TREATED WITH
5-CHLORO-o-TOLUIDINE

	CONTROL (UNTR) 11-1136	LOW DOSE 11-1134	HIGH DOSE 11-1132
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS NECROPSIED	20	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY**	20	49	49
INTEGUMENTARY SYSTEM			
*SUBCUT TISSUE	(20)	(50)	(50)
FIBROSARCOMA			1 (2%)
RESPIRATORY SYSTEM			
#LUNG	(20)	(48)	(48)
ALVEOLAR/BRONCHIOLAR ADENOMA	1 (5%)		1 (2%)
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(20)	(50)	(50)
MALIGNANT LYMPHOMA, NOS		1 (2%)	
CIRCULATORY SYSTEM			
NONE			
DIGESTIVE SYSTEM			
#LIVER	(20)	(49)	(49)
NEOPLASTIC NODULE		1 (2%)	
URINARY SYSTEM			
NONE			
ENDOCRINE SYSTEM			
#PITUITARY	(20)	(43)	(46)
CHROMOPHOBE ADENOMA	5 (25%)	4 (9%)	7 (15%)

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED
**EXCLUDES PARTIALLY AUTOLYZED ANIMALS

TABLE A2 (CONTINUED)

	CONTROL (UNTR) 11-1136	LOW DOSE 11-1134	HIGH DOSE 11-1132
CHROMOPHOBE CARCINOMA		2 (5%)	
MIXED TUMOR, BENIGN		1 (2%)	
#ADRENAL	(20)	(48)	(48)
PHEOCHROMOCYTOMA	1 (5%)		1 (2%)
#THYROID	(16)	(41)	(47)
FOLLICULAR-CELL CARCINOMA			2 (4%)
C-CELL ADENOMA	1 (6%)	1 (2%)	1 (2%)
C-CELL CARCINOMA	1 (6%)		5 (11%)
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(20)	(50)	(50)
ADENOCARCINOMA, NOS			1 (2%)
CYSTADENOMA, NOS	1 (5%)		
FIBROMA		1 (2%)	
FIBROADENOMA	1 (5%)	10 (20%)	5 (10%)
*PREPUTIAL GLAND	(20)	(50)	(50)
CYSTADENOMA, NOS		1 (2%)	
#UTERUS	(18)	(49)	(47)
ENDOMETRIAL STROMAL POLYP		4 (8%)	1 (2%)
#CERVIX UTERI	(18)	(49)	(47)
LEIOMYOMA			1 (2%)
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
NONE			

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE A2 (CONCLUDED)

	CONTROL (UNTR) 11-1136	LOW DOSE 11-1134	HIGH DOSE 11-1132
ALL OTHER SYSTEMS			
*MULTIPLE ORGANS	(20)	(50)	(50)
UNDIFFERENTIATED CARCINOMA		1 (2%)	
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	20	50	50
NATURAL DEATH@	1	8	4
MORIBUND SACRIFICE	4	2	
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	15	40	46
ANIMAL MISSING			
@ INCLUDES AUTOLYZED ANIMALS			
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	8	22	23
TOTAL PRIMARY TUMORS	11	27	26
TOTAL ANIMALS WITH BENIGN TUMORS	7	19	14
TOTAL BENIGN TUMORS	10	22	17
TOTAL ANIMALS WITH MALIGNANT TUMORS	1	4	9
TOTAL MALIGNANT TUMORS	1	4	9
TOTAL ANIMALS WITH SECONDARY TUMORS#			
TOTAL SECONDARY TUMORS			
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT		1	
TOTAL UNCERTAIN TUMORS		1	
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			

* PRIMARY TUMORS: ALL TUMORS EXCEPT SECONDARY TUMORS
\# SECONDARY TUMORS: METASTATIC TUMORS OR TUMORS INVASIVE INTO AN ADJACENT ORGAN

APPENDIX B

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MICE TREATED WITH 5-CHLORO-o-TOLUIDINE

TABLE B1
SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE MICE TREATED WITH
5-CHLORO-o-TOLUIDINE

	CONTROL (UNTR) 22-2135	LOW DOSE 22-2133	HIGH DOSE 22-2131
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS MISSING			2
ANIMALS NECROPSIED	20	50	48
ANIMALS EXAMINED HISTOPATHOLOGICALLY**	20	50	48
INTEGUMENTARY SYSTEM			
*SUBCUT TISSUE	(20)	(50)	(48)
HEMANGIOSARCOMA		7 (14%)	4 (8%)
RESPIRATORY SYSTEM			
#LUNG	(20)	(50)	(48)
ALVEOLAR/BRONCHIOLAR ADENOMA	2 (10%)	2 (4%)	1 (2%)
ALVEOLAR/BRONCHIOLAR CARCINOMA			1 (2%)
HEMANGIOSARCOMA, METASTATIC		1 (2%)	4 (8%)
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(20)	(50)	(48)
MALIGNANT LYMPHOMA, NOS		1 (2%)	1 (2%)
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE		1 (2%)	
#SPLEEN	(17)	(42)	(45)
HEMANGIOSARCOMA	1 (6%)	1 (2%)	3 (7%)
HEMANGIOSARCOMA, METASTATIC		1 (2%)	
CIRCULATORY SYSTEM			
#HEART	(18)	(47)	(48)
HEMANGIOSARCOMA, METASTATIC			2 (4%)
DIGESTIVE SYSTEM			
#LIVER	(20)	(50)	(47)
HEPATOCELLULAR ADENOMA		1 (2%)	2 (4%)

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED
**EXCLUDES PARTIALLY AUTOLYZED ANIMALS

TABLE B1 (CONTINUED)

	CONTROL (UNTR) 22-2135	LOW DOSE 22-2133	HIGH DOSE 22-2131
HEPATOCELLULAR CARCINOMA	4 (20%)	19 (38%)	25 (53%)
HEMANGIOSARCOMA	1 (5%)		
HEMANGIOSARCOMA, METASTATIC		1 (2%)	2 (4%)
URINARY SYSTEM			
#KIDNEY	(20)	(50)	(47)
HEMANGIOSARCOMA			1 (2%)
#PERIRENAL TISSUE	(20)	(50)	(47)
HEMANGIOSARCOMA		1 (2%)	
ENDOCRINE SYSTEM			
NONE			
REPRODUCTIVE SYSTEM			
*EPIDIDYMIS	(20)	(50)	(48)
HEMANGIOSARCOMA		2 (4%)	29 (60%)
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
*SKELETAL MUSCLE	(20)	(50)	(48)
HEMANGIOSARCOMA			1 (2%)
HEMANGIOSARCOMA, INVASIVE			1 (2%)
BODY CAVITIES			
*ABDOMINAL CAVITY	(20)	(50)	(48)
HEMANGIOSARCOMA, METASTATIC			1 (2%)

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE B1 (CONCLUDED)

	CONTROL(UNTR) 22-2135	LOW DOSE 22-2133	HIGH DOSE 22-2131
*MESENTERY	(20)	(50)	(48)
HEPATOCELLULAR CARCINOMA, METAST		1 (2%)	
ALL OTHER SYSTEMS			
NONE			
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	20	50	50
NATURAL DEATH@	1	8	24
MORIBUND SACRIFICE		1	1
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	19	41	23
ANIMAL MISSING			2
@ INCLUDES AUTOLYZED ANIMALS			
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	6	31	46
TOTAL PRIMARY TUMORS	8	35	68
TOTAL ANIMALS WITH BENIGN TUMORS	2	3	3
TOTAL BENIGN TUMORS	2	3	3
TOTAL ANIMALS WITH MALIGNANT TUMORS	5	29	45
TOTAL MALIGNANT TUMORS	6	32	65
TOTAL ANIMALS WITH SECONDARY TUMORS#		3	8
TOTAL SECONDARY TUMORS		4	10
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT			
TOTAL UNCERTAIN TUMORS			
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			
* PRIMARY TUMORS: ALL TUMORS EXCEPT SECONDARY TUMORS			
# SECONDARY TUMORS: METASTATIC TUMORS OR TUMORS INVASIVE INTO AN ADJACENT ORGAN			

TABLE B2
SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE MICE TREATED WITH
5-CHLORO-o-TOLUIDINE

	CONTROL (UNTR) 22-2136	LOW DOSE 22-2134	HIGH DOSE 22-2132
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS MISSING			6
ANIMALS NECROPSIED	20	50	43
ANIMALS EXAMINED HISTOPATHOLOGICALLY**	20	50	43
INTEGUMENTARY SYSTEM			
*SUBCUT TISSUE	(20)	(50)	(43)
HEMANGIOSARCOMA		6 (12%)	21 (49%)
RESPIRATORY SYSTEM			
#LUNG	(20)	(48)	(43)
HEPATOCELLULAR CARCINOMA, METAST			2 (5%)
ALVEOLAR/BRONCHIOLAR ADENOMA	1 (5%)		1 (2%)
ALVEOLAR/BRONCHIOLAR CARCINOMA			2 (5%)
HEMATOPOIETIC SYSTEM			
*MULTIPLE ORGANS	(20)	(50)	(43)
MALIG.LYMPHOMA, UNDIFFER-TYPE		1 (2%)	
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE		1 (2%)	
MALIG.LYMPHOMA, HISTIOCYTIC TYPE		1 (2%)	1 (2%)
LEUKEMIA,NOS	1 (5%)	1 (2%)	
*MEDIASTINUM	(20)	(50)	(43)
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE		1 (2%)	
#SPLEEN	(19)	(47)	(42)
HEMANGIOSARCOMA			1 (2%)
#MESENTERIC L. NODE	(14)	(34)	(27)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE			1 (4%)
#SMALL INTESTINE	(20)	(49)	(42)
MALIGNANT LYMPHOMA, NOS	1 (5%)		1 (2%)
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE		1 (2%)	1 (2%)
CIRCULATORY SYSTEM			
NONE			

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED
**EXCLUDES PARTIALLY AUTOLYZED ANIMALS

TABLE B2 (CONTINUED)

	CONTROL (UNTR) 22-2136	LOW DOSE 22-2134	HIGH DOSE 22-2132
DIGESTIVE SYSTEM			
#LIVER	(20)	(50)	(43)
HEPATOCELLULAR ADENOMA		2 (4%)	5 (12%)
HEPATOCELLULAR CARCINOMA		19 (38%)	26 (60%)
#DUODENUM	(20)	(49)	(42)
PAPILLARY CARCINOMA	1 (5%)		
URINARY SYSTEM			
#KIDNEY/CAPSULE	(20)	(48)	(43)
HEMANGIOSARCOMA, METASTATIC			1 (2%)
#URINARY BLADDER	(18)	(42)	(32)
HEMANGIOSARCOMA, INVASIVE			1 (3%)
ENDOCRINE SYSTEM			
NONE			
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(20)	(50)	(43)
ADENOMA, NOS			1 (2%)
#UTERUS	(20)	(48)	(39)
ENDOMETRIAL STROMAL POLYP		1 (2%)	
HEMANGIOSARCOMA, INVASIVE			2 (5%)
NERVOUS SYSTEM			
NONE			
SPECIAL SENSE ORGANS			
*HARDERIAN GLAND	(20)	(50)	(43)
ADENOMA, NOS		1 (2%)	
MUSCULOSKELETAL SYSTEM			
NONE			

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE B2 (CONTINUED)

	CONTROL (UNTR) 22-2136	LOW DOSE 22-2134	HIGH DOSE 22-2132
BODY CAVITIES			
*MEDIASTINUM	(20)	(50)	(43)
HEMANGIOSARCOMA, METASTATIC			1 (2%)
ALL OTHER SYSTEMS			
*MULTIPLE ORGANS	(20)	(50)	(43)
OSTEOSARCOMA, METASTATIC		1 (2%)	
THORACIC CAVITY			
HEMANGIOSARCOMA, METASTATIC			1
ANIMAL DISPOSITION SUMMARY			
ANIMALS INITIALLY IN STUDY	20	50	50
NATURAL DEATH@	2	7	12
MORIBUND SACRIFICE			1
SCHEDULED SACRIFICE			
ACCIDENTALLY KILLED			
TERMINAL SACRIFICE	18	43	31
ANIMAL MISSING			6

@ INCLUDES AUTOLYZED ANIMALS

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE B2 (CONCLUDED)

	CONTROL (UNTR) 22-2136	LOW DOSE 22-2134	HIGH DOSE 22-2132
TUMOR SUMMARY			
TOTAL ANIMALS WITH PRIMARY TUMORS*	4	31	38
TOTAL PRIMARY TUMORS	4	35	61
TOTAL ANIMALS WITH BENIGN TUMORS	1	4	7
TOTAL BENIGN TUMORS	1	4	7
TOTAL ANIMALS WITH MALIGNANT TUMORS	3	29	36
TOTAL MALIGNANT TUMORS	3	31	54
TOTAL ANIMALS WITH SECONDARY TUMORS#		1	5
TOTAL SECONDARY TUMORS		1	8
TOTAL ANIMALS WITH TUMORS UNCERTAIN- BENIGN OR MALIGNANT			
TOTAL UNCERTAIN TUMORS			
TOTAL ANIMALS WITH TUMORS UNCERTAIN- PRIMARY OR METASTATIC			
TOTAL UNCERTAIN TUMORS			

* PRIMARY TUMORS: ALL TUMORS EXCEPT SECONDARY TUMORS
\# SECONDARY TUMORS: METASTATIC TUMORS OR TUMORS INVASIVE INTO AN ADJACENT ORGAN

APPENDIX C

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN RATS TREATED WITH 5-CHLORO-o-TOLUIDINE

TABLE C1
SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE RATS TREATED WITH
5-CHLORO-o-TOLUIDINE

	CONTROL (UNTR) 11-1135	LOW DOSE 11-1133	HIGH DOSE 11-1131
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS NECROPSIED	20	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY**	20	50	50
INTEGUMENTARY SYSTEM			
NONE			
RESPIRATORY SYSTEM			
*NASAL CAVITY	(20)	(50)	(50)
KERATOSIS FOLLICULARIS		1 (2%)	
#LUNG/BRONCHIOLE	(20)	(49)	(50)
HYPERPLASIA, EPITHELIAL		1 (2%)	
#LUNG	(20)	(49)	(50)
CONGESTION, NOS		1 (2%)	
HEMORRHAGE		1 (2%)	
INFLAMMATION, NOS		1 (2%)	
INFLAMMATION, INTERSTITIAL		1 (2%)	
INFLAMMATION, ACUTE		1 (2%)	
PNEUMONIA, CHRONIC MURINE	6 (30%)	1 (2%)	6 (12%)
INFLAMMATION, CHRONIC	7 (35%)		
INFLAMMATION, CHRONIC FOCAL	1 (5%)		
FIBROSIS		1 (2%)	
HEMATOPOIETIC SYSTEM			
#BONE MARROW	(17)	(35)	(48)
HYPERPLASIA, HEMATOPOIETIC		1 (3%)	
#SPLEEN	(20)	(49)	(50)
CONGESTION, NOS		2 (4%)	6 (12%)
HEMOSIDEROSIS		2 (4%)	5 (10%)
ERYTHROPHAGOCYTOSIS		1 (2%)	
HYPERPLASIA, RETICULUM CELL		1 (2%)	

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED
**EXCLUDES PARTIALLY AUTOLYZED ANIMALS

TABLE C1 (CONTINUED)

	CONTROL (UNTR) 11-1135	LOW DOSE 11-1133	HIGH DOSE 11-1131
HEMATOPOIESIS	2 (10%)	2 (4%)	3 (6%)
MYELOPOIESIS		1 (2%)	
#LYMPH NODE	(17)	(42)	(35)
INFLAMMATION, HEMORRHAGIC			1 (3%)
HYPERPLASIA, RETICULUM CELL	1 (6%)		2 (6%)
#MESENTERIC L. NODE	(17)	(42)	(35)
HYPERPLASIA, RETICULUM CELL		1 (2%)	3 (9%)
HYPERPLASIA, LYMPHOID		1 (2%)	
CIRCULATORY SYSTEM			
#MYOCARDIUM	(20)	(50)	(50)
INFLAMMATION, FOCAL			1 (2%)
INFLAMMATION, ACUTE		1 (2%)	
INFLAMMATION, CHRONIC		1 (2%)	
FIBROSIS	2 (10%)	1 (2%)	1 (2%)
FIBROSIS, FOCAL		1 (2%)	2 (4%)
#ENDOCARDIUM	(20)	(50)	(50)
INFLAMMATION, ACUTE		1 (2%)	
DIGESTIVE SYSTEM			
#SALIVARY GLAND	(19)	(45)	(47)
ATROPHY, NOS		1 (2%)	1 (2%)
#LIVER	(20)	(48)	(50)
CONGESTION, NOS			1 (2%)
INFLAMMATION, ACUTE			1 (2%)
CIRRHOSIS, NOS		1 (2%)	
NECROSIS, NOS		1 (2%)	
METAMORPHOSIS FATTY		3 (6%)	11 (22%)
LIPOIDOSIS			1 (2%)
FOCAL CELLULAR CHANGE	1 (5%)	1 (2%)	
CYTOLOGIC DEGENERATION			1 (2%)
ANGIECTASIS		1 (2%)	1 (2%)
HEMATOPOIESIS			1 (2%)
#LIVER/CENTRILOBULAR	(20)	(48)	(50)
CONGESTION, NOS		1 (2%)	
#BILE DUCT	(20)	(48)	(50)
HYPERPLASIA, NOS	1 (5%)		2 (4%)

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE C1 (CONTINUED)

	CONTROL (UNTR) 11-1135	LOW DOSE 11-1133	HIGH DOSE 11-1131
HYPERPLASIA, FOCAL		2 (4%)	
#PANCREAS	(20)	(45)	(45)
FIBROSIS, DIFFUSE		1 (2%)	
ATROPHY, NOS			1 (2%)
#PANCREATIC ACINUS	(20)	(45)	(45)
ATROPHY, NOS	1 (5%)		4 (9%)
#ESOPHAGUS	(14)	(30)	(42)
HYPERPLASIA, NOS		1 (3%)	
#STOMACH	(20)	(50)	(50)
INFLAMMATION, ACUTE		1 (2%)	
#SMALL INTESTINE	(20)	(49)	(50)
ATROPHY, NOS	1 (5%)		
HYPERPLASIA, LYMPHOID	1 (5%)		10 (20%)
#LARGE INTESTINE	(20)	(49)	(49)
NEMATODIASIS	1 (5%)		3 (6%)
#COLON	(20)	(49)	(49)
INFLAMMATION, CHRONIC			1 (2%)
HYPERPLASIA, LYMPHOID			4 (8%)
URINARY SYSTEM			
#KIDNEY	(20)	(50)	(50)
INFLAMMATION, NOS	5 (25%)	2 (4%)	1 (2%)
INFLAMMATION, INTERSTITIAL		1 (2%)	1 (2%)
INFLAMMATION, CHRONIC	1 (5%)	3 (6%)	14 (28%)
GLOMERULONEPHRITIS, CHRONIC			5 (10%)
NEPHROPATHY		1 (2%)	
ARTERIOSCLEROSIS, NOS		1 (2%)	
NEPHROSIS, NOS	3 (15%)	3 (6%)	6 (12%)
ATROPHY, NOS		1 (2%)	
#KIDNEY/TUBULE	(20)	(50)	(50)
NEPHROSIS, NOS		2 (4%)	3 (6%)
ENDOCRINE SYSTEM			
#PITUITARY	(18)	(48)	(48)
DEGENERATION, NOS			1 (2%)

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE C1 (CONTINUED)

	CONTROL (UNTR) 11-1135	LOW DOSE 11-1133	HIGH DOSE 11-1131
HYPERPLASIA, CHROMOPHOBE-CELL			1 (2%)
DYSPLASIA, NOS			1 (2%)
#ADRENAL	(20)	(49)	(48)
CYST, NOS			1 (2%)
HEMORRHAGIC CYST	3 (15%)	2 (4%)	1 (2%)
INFARCT, NOS		1 (2%)	
HYPERPLASIA, NOS			1 (2%)
#ADRENAL MEDULLA	(20)	(49)	(48)
HEMORRHAGIC CYST	1 (5%)		
DEGENERATION, NOS		1 (2%)	
CALCIFICATION, NOS		1 (2%)	
HYPERPLASIA, NOS		1 (2%)	1 (2%)
#THYROID	(18)	(44)	(24)
HYPERPLASIA, NOS		1 (2%)	1 (4%)
HYPERPLASIA, C-CELL		1 (2%)	
#PANCREATIC ISLETS	(20)	(45)	(45)
FIBROSIS	1 (5%)		
REPRODUCTIVE SYSTEM			
#PROSTATE	(1)	(44)	(42)
INFLAMMATION, SUPPURATIVE			1 (2%)
INFLAMMATION, CHRONIC			1 (2%)
HYPERPLASIA, NOS	1 (100%)	2 (5%)	3 (7%)
HYPERPLASIA, EPITHELIAL			1 (2%)
HYPERPLASIA, CYSTIC			3 (7%)
*COAGULATING GLAND	(20)	(50)	(50)
HYPERPLASIA, NOS			1 (2%)
#TESTIS	(20)	(50)	(50)
DEGENERATION, NOS			1 (2%)
ATROPHY, NOS			2 (4%)
#TESTIS/TUBULE	(20)	(50)	(50)
DEGENERATION, NOS			1 (2%)
NERVOUS SYSTEM			
#BRAIN/MENINGES	(20)	(49)	(49)
INFLAMMATION, SUPPURATIVE	1 (5%)		

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE C1 (CONCLUDED)

	CONTROL (UNTR) 11-1135	LOW DOSE 11-1133	HIGH DOSE 11-1131
#BRAIN	(20)	(49)	(49)
NECROSIS, FOCAL			1 (2%)
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*PERITONEUM	(20)	(50)	(50)
INFLAMMATION, ACUTE			1 (2%)
INFLAMMATION, CHRONIC			1 (2%)
*PLEURA	(20)	(50)	(50)
INFLAMMATION, CHRONIC	1 (5%)		
ALL OTHER SYSTEMS			
NONE			
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED		1	

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE C2
SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE RATS TREATED WITH 5-CHLORO-o-TOLUIDINE

	CONTROL (UNTR) 11-1136	LOW DOSE 11-1134	HIGH DOSE 11-1132
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS NECROPSIED	20	50	50
ANIMALS EXAMINED HISTOPATHOLOGICALLY**	20	49	49
INTEGUMENTARY SYSTEM			
*SUBCUT TISSUE	(20)	(50)	(50)
FIBROUS DYSPLASIA		1 (2%)	
RESPIRATORY SYSTEM			
#TRACHEA	(17)	(45)	(46)
INFLAMMATION, NOS	1 (6%)		
#LUNG	(20)	(48)	(48)
ATELECTASIS	1 (5%)		
CONGESTION, NOS		1 (2%)	
INFLAMMATION, NOS		1 (2%)	
INFLAMMATION, INTERSTITIAL			1 (2%)
BRONCHOPNEUMONIA, ACUTE			1 (2%)
INFLAMMATION, ACUTE		1 (2%)	1 (2%)
PNEUMONIA, CHRONIC MURINE	1 (5%)	4 (8%)	3 (6%)
INFLAMMATION, CHRONIC		1 (2%)	
HYPERPLASIA, ADENOMATOUS	1 (5%)		
HEMATOPOIETIC SYSTEM			
#BONE MARROW	(12)	(37)	(44)
HYPERPLASIA, HEMATOPOIETIC			1 (2%)
#SPLEEN	(20)	(48)	(46)
CONGESTION, NOS	5 (25%)	5 (10%)	4 (9%)
INFLAMMATION, ACUTE			1 (2%)
HEMOSIDEROSIS	6 (30%)	5 (10%)	6 (13%)
ERYTHROPHAGOCYTOSIS			1 (2%)
HEMATOPOIESIS	4 (20%)	3 (6%)	4 (9%)
#LYMPH NODE	(13)	(35)	(44)
HYPERPLASIA, RETICULUM CELL	2 (15%)	2 (6%)	1 (2%)

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED
**EXCLUDES PARTIALLY AUTOLYZED ANIMALS

TABLE C2 (CONTINUED)

	CONTROL (UNTR) 11-1136	LOW DOSE 11-1134	HIGH DOSE 11-1132
#MESENTERIC L. NODE	(13)	(35)	(44)
HYPERPLASIA, RETICULUM CELL	1 (8%)	3 (9%)	3 (7%)
CIRCULATORY SYSTEM			
#MYOCARDIUM	(19)	(49)	(49)
FIBROSIS		1 (2%)	
FIBROSIS, FOCAL	1 (5%)	1 (2%)	1 (2%)
DIGESTIVE SYSTEM			
#LIVER	(20)	(49)	(49)
INFLAMMATION, FOCAL			1 (2%)
INFLAMMATION, ACUTE			1 (2%)
INFLAMMATION, GRANULOMATOUS		1 (2%)	
NECROSIS, FOCAL		1 (2%)	1 (2%)
METAMORPHOSIS FATTY	1 (5%)	11 (22%)	14 (29%)
LIPOIDOSIS		1 (2%)	1 (2%)
FOCAL CELLULAR CHANGE		4 (8%)	7 (14%)
CYTOLOGIC DEGENERATION			1 (2%)
HYPERPLASIA, NODULAR			1 (2%)
HYPERPLASIA, NOS		2 (4%)	1 (2%)
HEMATOPOIESIS	1 (5%)		
#BILE DUCT	(20)	(49)	(49)
INFLAMMATION, GRANULOMATOUS		2 (4%)	
HYPERPLASIA, NOS	1 (5%)	1 (2%)	3 (6%)
#PANCREAS	(18)	(47)	(47)
INFLAMMATION ACUTE AND CHRONIC			1 (2%)
NECROSIS, FAT		1 (2%)	
#PANCREATIC ACINUS	(18)	(47)	(47)
ATROPHY, NOS		1 (2%)	1 (2%)
#GASTRIC MUCOSA	(20)	(49)	(46)
HYPERPLASIA, NOS		1 (2%)	
#SMALL INTESTINE	(20)	(47)	(48)
ULCER, NOS	1 (5%)		
HYPERPLASIA, LYMPHOID	2 (10%)	7 (15%)	6 (13%)
#LARGE INTESTINE	(20)	(46)	(48)
NEMATODIASIS		2 (4%)	

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE C2 (CONTINUED)

	CONTROL (UNTR) 11-1136	LOW DOSE 11-1134	HIGH DOSE 11-1132
#COLON	(20)	(46)	(48)
HYPERPLASIA, LYMPHOID		2 (4%)	
URINARY SYSTEM			
#KIDNEY	(20)	(49)	(49)
GLOMERULONEPHRITIS, NOS			1 (2%)
INFLAMMATION, NOS	3 (15%)	6 (12%)	2 (4%)
INFLAMMATION, CHRONIC	1 (5%)	8 (16%)	5 (10%)
GLOMERULONEPHRITIS, CHRONIC		2 (4%)	
NEPHROSIS, NOS	2 (10%)	1 (2%)	5 (10%)
CALCINOSIS, NOS	1 (5%)		
#KIDNEY/TUBULE	(20)	(49)	(49)
NEPHROSIS, NOS		2 (4%)	2 (4%)
*URETER	(20)	(50)	(50)
CALCULUS, NOS	1 (5%)		
#URINARY BLADDER	(18)	(47)	(44)
INFLAMMATION, CHRONIC			1 (2%)
HYPERPLASIA, EPITHELIAL			1 (2%)
ENDOCRINE SYSTEM			
#PITUITARY	(20)	(43)	(46)
HEMORRHAGE	1 (5%)		
HEMORRHAGIC CYST	1 (5%)		
FIBROSIS	1 (5%)		
#ADRENAL	(20)	(48)	(48)
CYST, NOS			1 (2%)
HEMORRHAGIC CYST		1 (2%)	1 (2%)
LIPOIDOSIS		1 (2%)	
#ADRENAL CORTEX	(20)	(48)	(48)
HYPERPLASIA, NOS		1 (2%)	
#ADRENAL MEDULLA	(20)	(48)	(48)
CYST, NOS		1 (2%)	
#THYROID	(16)	(41)	(47)
CYST, NOS			1 (2%)

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE C2 (CONTINUED)

	CONTROL (UNTR) 11-1136	LOW DOSE 11-1134	HIGH DOSE 11-1132
HYPERPLASIA, ADENOMATOUS	1 (6%)		
HYPERPLASIA, C-CELL		2 (5%)	1 (2%)
#THYROID FOLLICLE	(16)	(41)	(47)
COLLAPSE			1 (2%)
REPRODUCTIVE SYSTEM			
*MAMMARY GLAND	(20)	(50)	(50)
HYPERPLASIA, NOS			1 (2%)
DYSPLASIA, NOS			1 (2%)
FIBROUS DYSPLASIA		1 (2%)	
#UTERUS	(18)	(49)	(47)
INFLAMMATION, SUPPURATIVE			1 (2%)
ABSCESS, NOS			1 (2%)
#UTERUS/ENDOMETRIUM	(18)	(49)	(47)
HYPERPLASIA, CYSTIC		3 (6%)	1 (2%)
#OVARY/PAROVARIAN	(18)	(49)	(47)
NECROSIS, FAT		1 (2%)	
#OVARY	(18)	(25)	(49)
CYST, NOS		1 (4%)	2 (4%)
FOLLICULAR CYST, NOS	1 (6%)		
NERVOUS SYSTEM			
#BRAIN	(20)	(48)	(47)
ABSCESS, NOS		1 (2%)	
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
NONE			
BODY CAVITIES			
*MESENTERY	(20)	(50)	(50)
NECROSIS, FAT		1 (2%)	

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE C2 (CONCLUDED)

	CONTROL (UNTR) 11-1136	LOW DOSE 11-1134	HIGH DOSE 11-1132
ALL OTHER SYSTEMS			
NONE			
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED	2	5	3
AUTO/NECROPSY/NO HISTO		1	1

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

APPENDIX D

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MICE TREATED WITH 5-CHLORO-o-TOLUIDINE

TABLE DI
SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE MICE TREATED WITH
5-CHLORO-o-TOLUIDINE

	CONTROL (UNTR) 22-2135	LOW DOSE 22-2133	HIGH DOSE 22-2131
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS MISSING			2
ANIMALS NECROPSIED	20	50	48
ANIMALS EXAMINED HISTOPATHOLOGICALLY**	20	50	48
INTEGUMENTARY SYSTEM			
#SKIN	(20)	(50)	(48)
PLASMACYTOSIS		1 (2%)	
#SUBCUT TISSUE	(20)	(50)	(48)
HEMANGIOMATOSIS		1 (2%)	
HEMORRHAGE			1 (2%)
RESPIRATORY SYSTEM			
#LUNG	(20)	(50)	(48)
PNEUMONIA, CHRONIC MURINE	2 (10%)	4 (8%)	4 (8%)
LEUKOCYTOSIS, NOS			1 (2%)
HEMATOPOIETIC SYSTEM			
#BONE MARROW	(19)	(47)	(45)
HYPERPLASIA, NOS		2 (4%)	
#SPLEEN	(17)	(42)	(45)
HEMATOPOIESIS		3 (7%)	1 (2%)
CIRCULATORY SYSTEM			
#HEART	(18)	(47)	(48)
PERIARTERITIS			1 (2%)
#MYOCARDIUM	(18)	(47)	(48)
INFLAMMATION, FOCAL		2 (4%)	2 (4%)
FIBROSIS, FOCAL		1 (2%)	

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED
**EXCLUDES PARTIALLY AUTOLYZED ANIMALS

TABLE D1 (CONTINUED)

	CONTROL (UNTR) 22-2135	LOW DOSE 22-2133	HIGH DOSE 22-2131
DIGESTIVE SYSTEM			
#LIVER	(20)	(50)	(47)
INFLAMMATION, FOCAL	2 (10%)		
LYMPHOCYTIC INFLAMMATORY INFILTR	1 (5%)		
ABSCESS, NOS		1 (2%)	
NECROSIS, NOS			1 (2%)
NECROSIS, FOCAL			2 (4%)
CYTOPLASMIC CHANGE, NOS		18 (36%)	11 (23%)
HYPERPLASIA, NODULAR			4 (9%)
HYPERPLASIA, NOS			2 (4%)
HYPERPLASIA, FOCAL		4 (8%)	1 (2%)
#PANCREAS	(19)	(45)	(44)
DILATATION/DUCTS		2 (4%)	
CYST, NOS			1 (2%)
CYSTIC DUCTS		1 (2%)	
PERIARTERITIS		1 (2%)	1 (2%)
#PANCREATIC ACINUS	(19)	(45)	(44)
ATROPHY, NOS	1 (5%)	3 (7%)	1 (2%)
#STOMACH	(20)	(49)	(46)
INFLAMMATION, ACUTE	1 (5%)		
#LARGE INTESTINE	(20)	(48)	(43)
NEMATODIASIS	9 (45%)	1 (2%)	1 (2%)
URINARY SYSTEM			
#KIDNEY	(20)	(50)	(47)
MINERALIZATION		2 (4%)	1 (2%)
HYDRONEPHROSIS			2 (4%)
GLOMERULONEPHRITIS, NOS		2 (4%)	11 (23%)
INFLAMMATION, FOCAL		1 (2%)	1 (2%)
SCAR		1 (2%)	
PERIARTERITIS			1 (2%)
HEMOSIDEROSIS		1 (2%)	9 (19%)
#KIDNEY/TUBULE	(20)	(50)	(47)
NECROSIS, NOS			1 (2%)
ENDOCRINE SYSTEM			
NONE			

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE D1 (CONTINUED)

	CONTROL (UNTR) 22-2135	LOW DOSE 22-2133	HIGH DOSE 22-2131
REPRODUCTIVE SYSTEM			
*SEMINAL VESICLE	(20)	(50)	(48)
INFLAMMATION, ACUTE			1 (2%)
#TESTIS	(18)	(50)	(46)
MINERALIZATION		1 (2%)	
#TESTIS/TUBULE	(18)	(50)	(46)
DEGENERATION, NOS		2 (4%)	4 (9%)
*EPIDIDYMIS	(20)	(50)	(48)
HEMANGIOMATOSIS		1 (2%)	
NERVOUS SYSTEM			
#BRAIN	(20)	(48)	(47)
CORPORA AMYLACEA	6 (30%)	24 (50%)	13 (28%)
#CEREBELLUM	(20)	(48)	(47)
HYDROCEPHALUS, NOS			1 (2%)
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
*SKELETAL MUSCLE	(20)	(50)	(48)
INFLAMMATION, NOS	1 (5%)		
BODY CAVITIES			
*MESENTERY	(20)	(50)	(48)
STEATITIS		1 (2%)	
INFLAMMATION ACUTE PUSTULAR		1 (2%)	
NECROSIS, FAT	1 (5%)		
ALL OTHER SYSTEMS			
OMENTUM			
PLASMA-CELL INFILTRATE		1	

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE D1 (CONCLUDED)

	CONTROL (UNTR) 22-2135	LOW DOSE 22-2133	HIGH DOSE 22-2131
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED	4	3	
ANIMAL MISSING/NO NECROPSY			2

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE D2
SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE MICE TREATED WITH 5-CHLORO-o-TOLUIDINE

	CONTROL (UNTR) 22-2136	LOW DOSE 22-2134	HIGH DOSE 22-2132
ANIMALS INITIALLY IN STUDY	20	50	50
ANIMALS MISSING			6
ANIMALS NECROPSIED	20	50	43
ANIMALS EXAMINED HISTOPATHOLOGICALLY**	20	50	43
INTEGUMENTARY SYSTEM			
NONE			
RESPIRATORY SYSTEM			
#LUNG	(20)	(48)	(43)
CONGESTION, NOS	1 (5%)	1 (2%)	
EDEMA, NOS		1 (2%)	
HEMORRHAGE			1 (2%)
PNEUMONIA, CHRONIC MURINE	4 (20%)	15 (31%)	3 (7%)
HISTIOCYTOSIS			1 (2%)
HEMATOPOIETIC SYSTEM			
#BONE MARROW	(19)	(48)	(37)
MYELOSCLEROSIS	7 (37%)	42 (88%)	32 (86%)
#SPLEEN	(19)	(47)	(42)
HEMOSIDEROSIS		1 (2%)	
HYPERPLASIA, LYMPHOID	2 (11%)	2 (4%)	
HEMATOPOIESIS		1 (2%)	
#BRONCHIAL LYMPH NODE	(14)	(34)	(27)
SCLEROSIS			1 (4%)
CIRCULATORY SYSTEM			
#HEART	(20)	(47)	(42)
MINERALIZATION		1 (2%)	
ABSCESS, NOS	1 (5%)		
#CARDIAC VALVE	(20)	(47)	(42)
MELANIN		1 (2%)	

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED
**EXCLUDES PARTIALLY AUTOLYZED ANIMALS

TABLE D2 (CONTINUED)

	CONTROL (UNTR) 22-2136	LOW DOSE 22-2134	HIGH DOSE 22-2132
DIGESTIVE SYSTEM			
*INTESTINAL TRACT	(20)	(50)	(43)
HYPERPLASIA, LYMPHOID			1 (2%)
*LIVER	(20)	(50)	(43)
HEMANGIOMATOSIS		1 (2%)	
HEMORRHAGIC CYST			1 (2%)
LYMPHOCYTIC INFLAMMATORY INFILTR	1 (5%)	1 (2%)	
NECROSIS, NOS		1 (2%)	
NECROSIS, FOCAL			1 (2%)
CYTOPLASMIC CHANGE, NOS		10 (20%)	7 (16%)
ANGIECTASIS		1 (2%)	
HEMATOPOIESIS		1 (2%)	
*PANCREAS	(20)	(45)	(41)
DILATATION/DUCTS			1 (2%)
*PANCREATIC ACINUS	(20)	(45)	(41)
ATROPHY, NOS			2 (5%)
*LARGE INTESTINE	(20)	(48)	(43)
NEMATODIASIS	1 (5%)		
URINARY SYSTEM			
*KIDNEY	(20)	(48)	(43)
MINERALIZATION		2 (4%)	
GLOMERULONEPHRITIS, NOS			3 (7%)
INFLAMMATION, FOCAL		1 (2%)	
LYMPHOCYTIC INFLAMMATORY INFILTR	1 (5%)		
INFLAMMATION, CHRONIC			1 (2%)
HEMOSIDEROSIS			2 (5%)
ENDOCRINE SYSTEM			
NONE			
REPRODUCTIVE SYSTEM			
*UTERUS	(20)	(48)	(39)
HEMANGIOMATOSIS		1 (2%)	

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE D2 (CONTINUED)

	CONTROL (UNTR) 22-2136	LOW DOSE 22-2134	HIGH DOSE 22-2132
CYST, NOS		9 (19%)	11 (28%)
PYOMETRA		4 (8%)	1 (3%)
#UTERUS/ENDOMETRIUM	(20)	(48)	(39)
CYST, NOS	2 (10%)	1 (2%)	
INFLAMMATION, NOS		1 (2%)	
INFLAMMATION, VESICULAR			1 (3%)
HYPERPLASIA, NOS	1 (5%)		
HYPERPLASIA, CYSTIC	3 (15%)	8 (17%)	1 (3%)
#OVARY	(15)	(36)	(26)
CYST, NOS	1 (7%)	4 (11%)	1 (4%)
PAROVARIAN CYST		2 (6%)	1 (4%)
INFLAMMATION, SUPPURATIVE			1 (4%)
NERVOUS SYSTEM			
#BRAIN/MENINGES	(20)	(48)	(41)
INFLAMMATION, NOS			1 (2%)
#BRAIN	(20)	(48)	(41)
INFLAMMATION, FOCAL			1 (2%)
CORPORA AMYLACEA	4 (20%)	12 (25%)	14 (34%)
SPECIAL SENSE ORGANS			
NONE			
MUSCULOSKELETAL SYSTEM			
*SKELETAL MUSCLE	(20)	(50)	(43)
INFLAMMATION, ACUTE	1 (5%)		
PARASITISM	1 (5%)		
BODY CAVITIES			
*MESENTERY	(20)	(50)	(43)
MINERALIZATION			1 (2%)
ALL OTHER SYSTEMS			
NONE			

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE D2 (CONCLUDED)

	CONTROL (UNTR) 22-2136	LOW DOSE 22-2134	HIGH DOSE 22-2132
SPECIAL MORPHOLOGY SUMMARY			
NO LESION REPORTED			1
ANIMAL MISSING/NO NECROPSY			6
AUTOLYSIS/NO NECROPSY			1

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

Review of the Bioassay of 5-Chloro-*o*-Toluidine* for Carcinogenicity
by the Data Evaluation/Risk Assessment Subgroup
of the Clearinghouse on Environmental Carcinogens

October 25, 1978

The Clearinghouse on Environmental Carcinogens was established in May, 1976, in compliance with DHEW Committee Regulations and the Provisions of the Federal Advisory Committee Act. The purpose of the Clearinghouse is to advise the Director of the National Cancer Institute (NCI) on its bioassay program to identify and to evaluate chemical carcinogens in the environment to which humans may be exposed. The members of the Clearinghouse have been drawn from academia, industry, organized labor, public interest groups, and State health officials. Members have been selected on the basis of their experience in carcinogenesis or related fields and, collectively, provide expertise in chemistry, biochemistry, biostatistics, toxicology, pathology, and epidemiology. Representatives of various Governmental agencies participate as ad hoc members. The Data Evaluation/Risk Assessment Subgroup of the Clearinghouse is charged with the responsibility of providing a peer review of reports prepared on NCI-sponsored bioassays of chemicals studied for carcinogenicity. It is in this context that the below critique is given on the bioassay of 5-Chloro-*o*-toluidine for carcinogenicity.

The reviewer for the report on the bioassay of 5-Chloro-*o*-toluidine said that, under the conditions of test, the compound was carcinogenic in both sexes of treated mice and that in rats the evidence suggested a carcinogenic effect but was not conclusive. He added that the results from the bioassay suggest that 5-Chloro-*o*-toluidine would pose a potential carcinogenic risk for humans.

In response to a question, a Program staff member commented that the incidence of adrenal tumors in treated male rats was not statistically significant based on a comparison with matched control animals.

There was no objection to a recommendation that the report on the bioassay of 5-Chloro-*o*-toluidine be accepted as written.

Clearinghouse Members Present:

Arnold L. Brown (Chairman), University of Wisconsin Medical School
Joseph Highland, Environmental Defense Fund
William Lijinsky, Frederick Cancer Research Center
Henry Pitot, University of Wisconsin Medical Center
Verne A. Ray, Pfizer Medical Research Laboratory
Kenneth Wilcox, Michigan State Health Department

* Subsequent to this review, changes may have been made in the bioassay report either as a result of the review or other reasons. Thus, certain comments and criticisms reflected in the review may no longer be appropriate.

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